MITRAGYNA SPECIOSA: A SYSTEMATIC REVIEW OF ITS PHARMACOLOGY AND SURVEY OF ITS USE IN THE NORTHERN STATES OF MALAYSIA

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Abstract

Mitragyna speciosa (MS) belongs to *Rubiaceae* family and is a tree native to countries in South East Asia. The consumption of *Mitragyna speciosa* (MS) for its psychoactive effects is widely reported amongst people in the villages in Thailand and Malaysia even though its use is illegal. Yet, little is known about its properties and pattern of use. The first part of the study aimed to examine MS pharmacological properties using systematic review methodology. The second part aimed to examine the prevalence, patterns of MS use as well as explored its potential to cause dependence in a cross-sectional study.

For the systematic review on the pharmacological properties of MS, comprehensive literature searches were carried out for articles on MS published from inception to February 2012 with no restriction on language. Thirty-one electronic databases, including the MEDLINE, TRIPDATABASE and BIOMED CENTRAL were searched. Only animal studies examining the pharmacological properties of MS or its compound were included while studies on the pharmacokinetic, toxicology and cell studies were excluded. Two review authors independently screened potentially eligible studies and extracted the data. A total of 44 studies were included in the review. Most of the studies examined the antinociceptive effects of MS (20 studies) and contractility of smooth muscle (14 studies). Other pharmacological effects examined included effects on bioamines levels, behavioural and the anti-inflammatory effects. It appears that mitragynine and 7-hydroxymitragynine, the two active constituents of MS have antinociceptive properties and that they act through μ- and δ-supraspinal opioid receptors.

In the cross-sectional study, we used both convenience and snowball-sampling methods to recruit participants in a border town between two northern states in Malaysia. Face-to-face interviews were conducted with the use of a structured questionnaire on 562 respondents who gave oral consent to participate in the study. The response rate was 91%. The majority of the respondents (88%) reported daily use of MS. The main mode of using MS was by drinking the MS extract as tea (90%). The mean age of starting MS use was 28.3 (SD = 8.1) years. A variety of reasons were given for using MS including for social and recreational needs, stamina and physical endurance, pain relief and improved sexual performance. Despite its reported usefulness in weaning off opiate addiction, 460 (87%) admitted they were not able to stop using MS. Only education level had a statistically significant association with the ability to stop or not stop the use of MS ($\chi2 = 31.0$, df = 1, $p < 0.001$). Significantly higher proportions of those with a lower education level (38%) were able to stop using MS compared to respondents with a higher education level (10%).

The study provides important information on the pharmacological effects of MS, its pattern of use and effects and its potential to cause addiction, as there has been growing interest in MS as evidenced by the number of advertisements for its sale on the Internet. Future study is required to explore its psychological and social impact on users.

Keywords: Ketum, kratom, biak-biak, Malaysia, addict, opiate.

Abstrak

Mitragyna speciosa (MS) tergolong dalam keluarga famili *Rubiaceae* dan adalah pokok yang berasal dari negara-negara di Asia Tenggara. Penggunaan *Mitragyna speciosa* (MS) untuk kesan psikoaktif dilaporkan secara meluas di kalangan masyarakat di kampung-kampung di Thailand dan Malaysia walaupun penggunaannya telah diharamkan. Namun hanya sedikit yang diketahui tentang sifat dan corak penggunaannya. Bahagian pertama kajian ini menumpukan kepada khasiat farmakologi MS dengan menggunakan metodologi *systematic review*. Bahagian kedua pula memeriksa kelaziman, corak pengunaan MS serta meneroka potensi ketagihan dalam *cross-sectional study*.

Untuk kajian *systematic review* ke atas sifat farmakologi MS, carian literatur yang menyeluruh telah dijalankan tentang artikel MS yang diterbitkan sejak tarikh terawal hingga Februari 2012 tanpa mengambil kira sebarang kekangan terhadap bahasa. Carian telah dilakukan ke atas 31 pangkalan data elektronik, termasuk MEDLINE, TRIPDATABASE dan BIOMED CENTRAL. Hanya penyelidikan terhadap haiwan yang menggunakan bahan kimia dari MS atau ektraksi MS diterima dalam *systematic review* ini. Kajian MS tentang farmakokinetik, toksikologi dan kajian sel tidak diterima. Dua penulis menilai dan mengektrak data secara individu terhadap kajian yang berpotensi untuk diterima. Sebanyak 44 kajian telah dimasukkan dalam kajian ini. Kebanyakan kajian meneliti kesan *antinociceptive* MS (20 kajian) dan kesan pengecutan otot licin (14 kajian). Lain-lain kesan farmakologi dikaji termasuk kesan ke atas tahap bioamines, tingkah laku dan kesan anti-radang. Ternyata bahawa mitragynine dan 7-hydroxymitragynine, kedua-dua komponen-komponen aktif MS mempunyai ciri-ciri *antinociceptive* dan mereka bertindak melalui reseptor opioid supraspinal μ dan δ.

Dalam *cross-sectional study*, kami menggunakan dua kaedah iaitu *convenient sampling* dan *snow-ball sampling* untuk mendapatkan responden di sempadan bandar antara dua negeri-negeri utara di Malaysia. Temuramah secara bersemuka telah dijalankan dengan menggunakan borang soal selidik berstruktur ke atas 562 responden yang memberikan persetujuan secara lisan untuk mengambil bahagian dalam kajian. Kadar maklum balas ialah 91%. Majoriti responden (88%) melaporkan penggunaan harian MS. Mod utama menggunakan MS adalah dengan meminum ekstrak MS sebagai teh (90%). Min umur memulakan penggunaan MS adalah 28.3 (SD = 8.1) tahun. Pelbagai alasan telah diberikan untuk menggunakan MS termasuk untuk keperluan sosial dan rekreasi, stamina dan ketahanan fizikal, melegakan kesakitan dan prestasi seksual yang lebih baik. Walaupun penggunaan dilaporkan untuk menyapih ketagihan *opiate*, 460 (87%) mengakui mereka tidak mampu untuk berhenti menggunakan MS. Hanya tahap pendidikan mempunyai hubungan yang ketara secara statistik dengan keupayaan untuk berhenti atau tidak berhenti menggunakan MS ($\chi2 = 31.0$, df = 1, $p <0.001$). Terdapat perbezaan yang ketara ke atas nisbah yang mempunyai tahap pendidikan yang lebih rendah (38%) mampu untuk berhenti menggunakan MS berbanding responden dengan tahap pendidikan yang lebih tinggi (10%).

Kajian ini menyediakan maklumat penting mengenai kesan-kesan farmakologi MS, corak penggunaan dan kesan dan potensi yang menyebabkan ketagihan, kerana terdapat peningkatan minat terhadap penggunaan MS seperti yang dibuktikan oleh bilangan iklan penjualannya di Internet. Kajian lanjut perlu dilakukan untuk meneroka kesan psikologi dan sosial kepada pengguna.

Kata kunci: Ketum, kratom, biak-biak, Malaysia, penagih, *opiate*.

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Kamarudin Bin Ahmad

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List of abbreviations and symbols

-	not available
?	unclear
√	compliance to criteria
5-HT_{2A}	5-Hydroxytryptamine 2A
6-OHDA	6-hydroxydopamine
AADK	National Anti-Drug Agency
AcOH	acetic acid
ATP	adenosine triphosphate
CAMARADES	Collaborative Approach to Meta Analysis and Review of Animal Data in Experimental Studies
CCK	cholecystokinin
DAMGO	D-Ala^2, N-$MePhe^4$ Gly-ol^5]-enkephalin
DAST-10	Drug Abuse Screening Test-10
ddY mice	Deutschland, Denken, and Yoken mice.
DPDPE	D-Pen2, D-Pen5-enkephalin
EEG	electroencephalography
etc.	et cetera
i.c.v	intracerebroventricular
i.g.	intragastric
i.p.	intraperitoneal
ICR mice	imprinting control region mice
KA	Kamarudin Ahmad
kg	kiligram
mg	milligram
MS	*Mitragyna speciosa*
n-BuOH	n-Butanol
NorBNI	norbinaltorphimine
ORL1	opioid receptor-like 1
p.o.	oral
PAIs	Privileged Access Interviewers
p-CPA	para-cholorophenalanine
p-CPA	p-chlorophenlalanine
RM	Ringgit Malaysia
s.c.	subcutaneous
SD	standard deviation
U69593	(5α,7α,8β)-(+)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide
USD	United States of American dollar
ZA	Zoriah Aziz
β-FNA	β-funaltrexamine

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List of published abstracts and journal publications from this study

a) Conference abstracts

Ahmad, K., & Aziz, Z. (2011). P044 *A systematic review on the central nervous system effects of Mitragyna speciosa alkoloids in animal studies.* Paper presented at the 25th Scientific Meeting of the Malaysian Society of Pharmacology and Physiology, Advances in Drug Development for a Better Well-being., Universiti Putra Malaysia, p.126.

Ahmad, K., Aziz, Z., & Mohamad, K. (2011). OP2 *A survey of Mitragyna speciosa use at the border town of Kedah and Perlis.* Paper presented at the 2nd Annual Health Conference 2011, Global Healthcare: Differing Perspectives and Dimensions in Healthcare, Kuala Lumpur, p.32.

b) Manuscript published in Journal of Ethnopharmacology

Ahmad, K., Aziz, Z., Mitragyna speciosa use in the Northern States of Malaysia: a cross-sectional study, Journal of Ethnopharmacology (2011), doi:10.1016/j.jep.2012.03.009

1. BACKGROUND

1.1 General introduction

Mitragyna speciosa (MS) belongs to the *Rubiaceae* family, grows well in swampy areas, and is native to countries in South-East Asia. It grows wild mainly in Thailand, Malaysia, Indonesia and Papua New Guinea and it is cultivated mostly for its leaves. The leaves are either prepared as a drink, chewed when freshly picked, or smoked when dried. In Thailand, MS is commonly known as “Kratom” while in Malaysia as “Biak-biak” or “Ketum.” Currently, out of more than 40 alkaloids identified in MS (Shellard, 1974), four alkaloids namely mitragynine, 7-hydroxymitragynine, corynantheidine and speciociliatine appear to act on opioid receptor (Takayama et al., 2002). Mitragynine, which accounts for about 66% of the total alkaloids, is the dominant alkaloid and is exclusive to MS (Ponglux et al., 1994; Shellard, 1974). It does not appear to have psychedelic activity (Matsumoto et al., 1997) although it has a chemical structure resembling a psychedelic rather than an opiate (Jansen and Prast, 1988a).

Burkill (1935) first recorded the use of MS in Malaya (present day Peninsula Malaysia) as a wound poultice, cure for fever and a substitute for opium, when opium was not available or not affordable. Traditionally MS has been used to wean addicts off heroin addiction, to treat diarrhoea, to improve blood circulation and to treat diabetes (Chan et al., 2005). However, there are also reports that the leaves are mainly used as a stimulant to enable labourers to endure physical fatigue (Jansen and Prast, 1988b). The first case of addiction to MS was first reported in 1957 and a case series of addiction was published in 1975 (Suwanlert, 1975; Thuan, 1957). Typical withdrawal symptoms reported include hostility, aggression, excessive tearing, inability to work, aching of muscles, bones, and jerky limb movements; whereas long-term use has been associated with anorexia, weight loss, and insomnia (Suwanlert, 1975).

In Malaysia, MS is widely available in the Northern States of Malaysia namely Kedah and Perlis that share a common border with Thailand and it is widely sold as drinks and teas (Chan et al., 2005). The drinks are easily available in the villages and are cheap. In 2003, the Malaysian government listed Mitragynine, the major alkaloid of MS in the First Schedule and the Third Schedule of Psychotropic substances in the Poisons Act 1952. Under this Act a person found to possess or sell MS leaves or other MS preparations such as drinks and teas containing mitragynine may be fined a maximum penalty of RM 10,000 (USD 3,300) a four-year jail sentence or both (Poison Act 1952, 2006). With the introduction of this restriction, the authorities have destroyed many trees which could mean a loss in the world's biodiversity as the species is found only in very few countries (Chan et al., 2005). MS is now believed to have the potential to be used as a medicinal product because of its antinociceptive properties, which have been reported to be somewhat better than opiates in animal studies (Matsumoto et al., 2005).

The use of MS in Malaysia has been widely debated. There are contradictory reports regarding the psychoactive properties of MS, claiming it to be both a narcotic and a stimulant; two effects which are generally regarded as opposite. It has been described that the leaves were abused as an alternative to narcotics (Vicknasingam et al., 2010). However, there is very little published information on the demographic of the user and their experiences with MS and there are many conflicting report on the effects of MS.

1.2 Justification of study

Although laboratory studies have provided useful data on the chemical and pharmacological properties of MS, the pharmacological findings were often contradictory. For example, several animal studies reported that MS has stimulant effects. Yet, other studies found that it has narcotic effects. In view of this contradiction, a systematic review to systematically examine the pharmacological effects of MS was warranted.

Reports from the National Anti-Drug Agency of Malaysia indicated that MS is being widely abused and it causes addiction problem (AADK, 2008). It was reported that many addicts have used MS as a substitute for narcotics, when narcotics were not available or not affordable. All these reports had been largely anecdotal. Little data are currently available on the pattern of its use and its effects as well as its potential to cause addiction. The present study therefore seeks to document the patterns, reasons, effects and correlates of MS usage among Malaysians. The need to document the use of MS is important because over the past decade there has been a growing interest in MS as evidenced by the number of advertisement for its sale on the internet (Schmidt et al., 2010). Given its increasing popularity on the internet, it is likely that the abuse of MS would become widespread in the future.

1.3 Aim of the study

This study consists of two parts focusing on two different aspects of MS.

The first part dealt with a systematic review on the pharmacology of MS. The specific objectives for the systematic review were to:

(a) search and identify all pharmacological investigation of MS in animal studies

(b) describe the characteristics of studies examining the pharmacological properties of MS

The second part deals with a survey on the use of MS among users in the border town between Kedah and Perlis. The specific objectives for this survey were to:

(a) explore the reasons for the use of *Mitragyna speciosa*

(b) determine the demographic correlates of use of *Mitragyna speciosa*

(c) examine other use of substance abuse among Mitragyna speciosa users

(d) explore the effects of *Mitragyna speciosa* experienced among the users

(e) examine the potential of dependence on MS

2. PART I: SYSTEMATIC REVIEW ON THE PHARMACOLOGY OF *MITRAGYNA SPECIOSA*

2.1 INTRODUCTION

2.1.1 Chemical constituents

More than twenty kinds of Corynanthe type alkaloids, including oxindole derivatives were isolated from various samples of MS from Thailand (Shellard et al., 1978a; Shellard et al., 1978b; Shellard et al., 1978c). Mitragynine was obtained from the young leaves of MS as the major constituent (66.2% based on the crude base) along with its analogues, speciogynine, speciociliatine, paynantheine and 7α-hydroxy-7H-mitragynine.

In Malaysians MS, other minor indole alkaloids found are 3,4-dehydro derivative of mitragynine, mitragynaline, corynantheidaline, mitragynalinic acid, and corynantheidaline acid, (Houghton et al., 1991) while in Thai MS the alkaloids are mitralactonal, mitrasulgynine and 3,4,5,6-tetradehydromitragynine (Takayama et al., 1998). Compared to Thai MS the methanolic extract of the mature leaves of MS from Malaysia have five similar alkaloids mitragynine, speciogynine, speciociliatine, paynantheine and 7-hydroxymitragynine (Takayama, 2004). The yield of mitragynine for Malaysian MS is much less than the Thai MS (Takayama, 2004).

2.1.2 Pharmacology

The pharmacological effects of MS have been attributed to various constituents of MS. For example, Macko et al. (1972) reported that the main alkaloid mitragynine showed antinociceptive and antitussive activities. They suggested that non-opioid systems are involved in the activity of mitragynine because nalorphine, an opioid receptor antagonist, failed to antagonise the antinociceptive activity of orally administered mitragynine in the tail-flick test in rats.

Mitragynine and 7-hydroxymitragynine was found to inhibit vascular smooth muscle of guinea-pig's ileum (Watanabe et al., 1997; Matsumoto et al., 2004; Matsumoto et al., 2006a) and mouse vas deferens (Matsumoto et al., 2005a; Matsumoto et al., 2006a). This inhibitory activity which delay gastrointestinal transit probably explained the constipation effect reported in MS user (Matsumoto et al., 2006a,c; Chittrakarn et al., 2008; Mossadeq et al., 2009b). MS showed some spasmogenic effects on guinea-pig's ileum in low dose but exhibited spasmolytic effects at higher dose. These effects could probably explain its use in human to relieve both diarrhoea and constipation (Mossadeq et al., 2009b; Mossadeq et al., 2011).

Another intriguing pharmacological effect is its stimulant properties. MS has been reported to be used by manual labourers to increase work productivity due to this effect (also called "coca-like" effects) (Suwanlert, 1975; Babu et al., 2008). Thus, MS is considered unusual because of its dual properties: as stimulant and as depressant (Abdullah, 1985). However, Farah Idayu et al. (2011) suggested that MS does not act like psycho-stimulant agent because it does not affect locomotors' activity; instead MS extract showed antidepressant effect in animal model. It is probable, that the conflicting effects were observed because of the presence of other chemicals or that MS exerts its effect on other system or pathways. Because of these contradictions, a systematic review was needed to review the pharmacological effects of MS.

2.1.3 Rationale of systematic review and its advantages

Several non-systematic reviews on MS are available; however this study is the first to examine the evidence by using systematic review approach. Systematic review is more superior to non-systematic review because it provides clear review objectives, involves comprehensive literature search and pre-specified inclusion and exclusion criteria. It is conducted with scientific rigour unseen in other reviews by detailing comprehensive searching for all the evidence and the assessment of the quality of the included studies in the review. Every decision made in the review is transparent. Thus, compared to non-systematic review available, bias in the finding of a systematic review is greatly minimised.

Additionally the systematic review could highlight conflicting evidence among studies, identify the best methods to conduct future animal study and save money by indicating when enough research has been done. Systematic review also helps to prevent duplicate studies or unnecessary animal trials.

2.1.4 Aim and justification of study

The aims of this study were to present the pharmacological effects of MS and highlight discrepancies among the data. The analysis would yield data on the pharmacological effects of MS in animals and identify weaknesses in current studies to guide future research to conduct better study in exploring the pharmacological activities of MS.

2.2 METHODS

2.2.1 Search strategy

Studies reporting the use of MS extracts, mitragynine and 7-hydroxymitragynine in an animal model were identified by searching 20 electronic databases **(Appendix A)** including the MEDLINE, TRIPDATABASE, Science Direct®, NEXTBIO and BIOMED CENTRAL. The search was from inception to February 2012 with no limit on language. We also performed hand search, and contacted experts in the area to identify additional studies. Editorial comments and narrative reviews were searched to cross-check their references for further study identification. The search strategies used are as shown in **(Appendix B)**. Titles obtained were screened independently by two reviewers Kamarudin Ahmad (KA) and Zoriah Aziz (ZA) to identify studies that meet the inclusion and exclusion criteria.

2.2.2 Inclusion and exclusion criteria

We included animal studies, which adequately report on any pharmacological effects of MS extract or any specific compounds extracted from MS. We excluded studies which examined the chemistry, pharmacokinetic profile, toxicology of MS or its constituents and cell studies. Studies which designed or synthesised new compound from MS were also excluded.

2.2.3 Data extraction

One reviewer (KA) searched titles and abstracts of citations on animal research involving MS using specific search strategy as detailed in section 2.2.1. Full-text articles were retrieved if at least one of the two reviewers believed it might be relevant. The two reviewers (KA, ZA) independently reviewed the full-text articles using the same criteria. The data were extracted by using a standardised data extraction form **(Appendix C)**. Any disagreement was resolved by consensus between the two reviewers.

2.2.4 Methodological quality

We assessed the susceptibility to bias of each publication with a standardised checklist named as Collaborative Approach to Meta Analysis and Review of Animal Data in Experimental Studies (CAMARADES) (Macleod et al., 2004). CAMARADES consists of 10 criteria as in **(Appendix D)** which are: peer-reviewed publication; statement of temperature control; random allocation to treatment or control; blinded to treatment; blinded to assessment of outcome; use of anaesthetic without significant intrinsic neuroprotective activity; suitable animal model (example: aged, diabetic, hypertensive or healthy); sample size calculation; compliance with animal welfare regulations; and statement of potential conflict of interests.

2.3 RESULTS AND DISCUSSIONS

2.3.1 Search result

The initial literature search yielded 1137 citations (Figure 2.1). We screened 912 titles and abstracts after removing 225 duplicates. We excluded a further 861 citations because they did not meet our inclusion criteria. These were reports of surveys, studies on MS chemistry, pharmacokinetic profile, reviews, letters, commentary and studies on cells.

We retrieved 51 full-text articles for further evaluation, and a further seven studies were excluded for various reasons. The reasons for exclusion are as in **(Appendix E)**. A total of 44 articles were included in this systematic review.

To our knowledge, this is the first systematic review using systematic review methodology on the pharmacology of MS that clearly defined inclusion and exclusion criteria and critically appraised the literature. There is a possibility of missing studies due to under reporting of negative studies or studies published in other languages even though we have made every effort to minimise this by undertaking a comprehensive search of the published and unpublished literature. Furthermore, studies on herbs are not always indexed in databases (Beahler et al., 2000; Whiting et al., 2008; Slobogean et al., 2009). It is a well known fact that in conducting any systematic review, tracing all relevant studies was considered difficult (Yoon et al., 2006; Whiting et al., 2008).

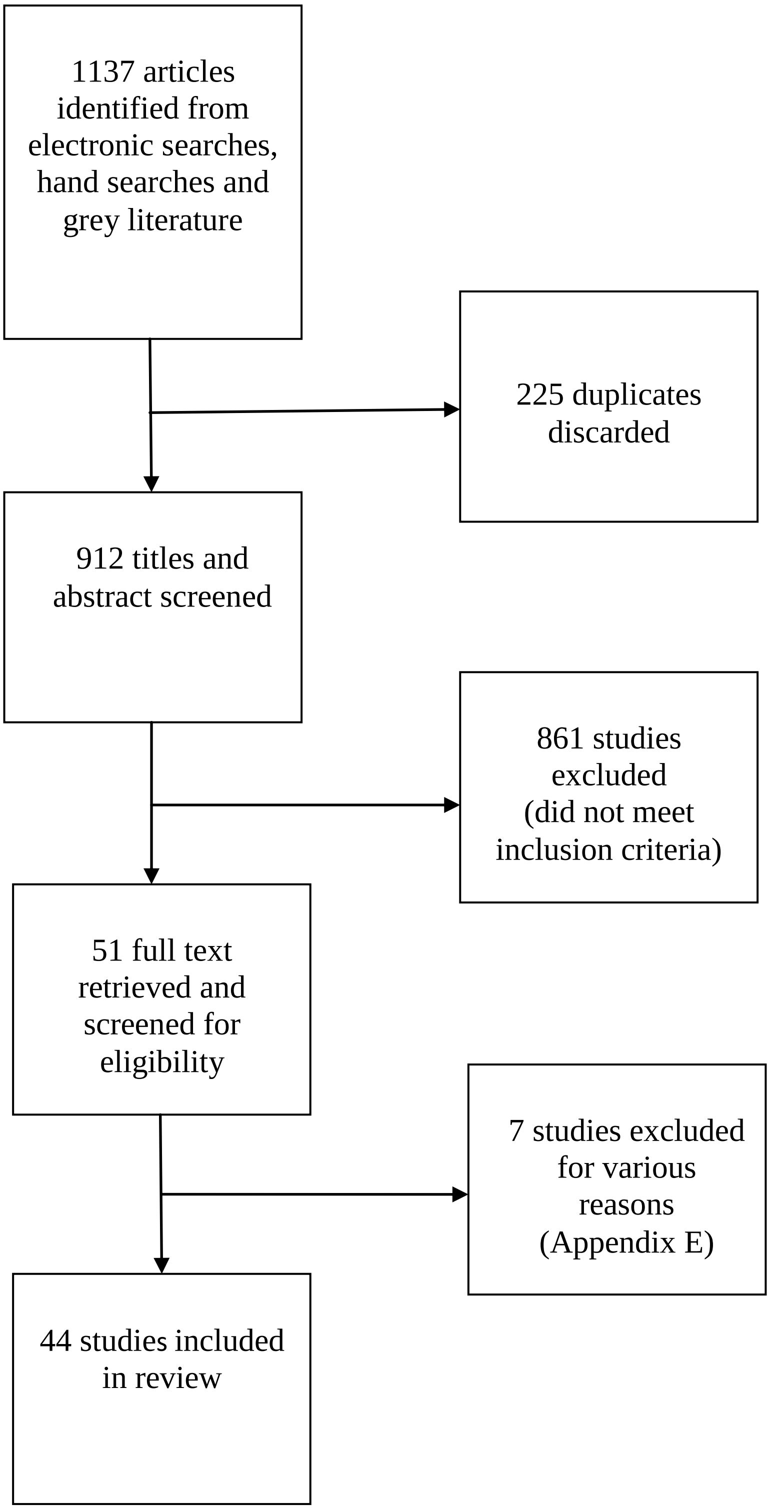
1137 articles identified from electronic searches, hand searches and grey literature
225 duplicates discarded
912 titles and abstract screened
861 studies excluded (did not meet inclusion criteria)
51 full text retrieved and screened for eligibility
7 studies excluded for various reasons (Appendix E)
44 studies included in review

Fig. 2.1: Flow diagram for study selection.

2.3.2 Characteristic of studies

A total of 44 animal studies assessing the pharmacological activities of MS or its constituents met our inclusion criteria. A variety of effects was examined involving many different test such as ileum contractility tests, tail-flick tests and sleep-time induced by phenobarbitone tests. Several studies examined either more than one MS constituents or more than one pharmacological properties. The most commonly used constituents examined in the pharmacological studies were mitragynine (21 studies) and 7-hydroxymitragynine (7 studies). Summary of the characteristic of all included studies are as in Table 2.1.

Table 2.1: Summary of study characteristics

Study	Animal(s)	Test(s)
Grewal, 1932a	Rabbit Guinea-pig Cat Frog (species not specified)	Local anaesthetic Peripheral nerves Contractility Cardiovascular
Macko et al., 1972	Sprague-Dawley rat Dog Cat Mice (species not specified)	Cardiovascular Antitussive D'Amour and Smith and Randall and Selitto Hardy, Wolff and Goodel Hot plate Nalorphine antagonism Charcoal meal Ileum contractility
Abdullah, 1985	Mice (species not specified)	Co-ordination (incline plane)
Hashim, 1987	Swiss Webster mice Wistar rats	Hot-plate Tail-flick Dependence
Looi, 1990	Mice (*Mus Musculus*)	Sleep-time Induce by Pentobarbitone
Dharmaraj, 1991	Mice (*Mus Musculus*)	Tail-flick Serotonin Assay
Inayat Hussain, 1991	Mice (*Mus Musculus*)	Noradrenalin Assay Tail-flick
Shahar et al., 1991	Mice (*Mus Musculus*) Guinea pig (*Cavia porcerlus*) Rat (*Rattus norvegicus*)	Tail-flick Ileum contractility Vas Deferens contractility
Ahmad, 1992	Rat (species not specified)	Tail-flick

Table 2.1, continued

Study	Animal(s)	Test(s)
Husain, 1993	Albino mice (species not specified)	Tail-flick Noradrenalin Assay
Aziz, 1993	Wistar rat	Drug Discrimination
Matsumoto et al., 1996a	ddY-mice	Hot-plate and tail-pinch
Matsumoto et al., 1996b	ddY mice	Hot-plate and tail-pinch
Matsumoto et al., 1997	ddY-mice	5-Methoxy-N,N-dimethyltryptamine-induced head twitch and spontaneous motor activity
Watanabe et al., 1997	Albino guinea-pigs (Dunkin-Hartley)	Contractility
Abushwereb, 1998	Wistar rats Albino mice	Biogenic Amines Assay Force Swimming Open field Plus-maze
Idid et al., 1998	Albino Mice	Writhing Hot tail-flick Cold tail-flick
Thongpradichote et al., 1998	ddY-mice	Hot-plate Tail-pinch
Takayama et al., 2000	Guinea-pig ileum (species not specified)	Contractility
Latif et al., 2002	Sprague Dawley rats	Biogenic Amines Assay

ddY-mice = Deutschland, Denken, and Yoken mice

Table 2.1, continued

Study	**Animal(s)**	**Test(s)**
Takayama et al., 2002	Albino Dunkin-Hartley guinea-pig	Contractility
Tsuchiya et al., 2002	Wistar rats	Gastric acid output
Matsumoto et al., 2004	ddY Mice	Hot tail-flick Tail-flick
Horie et al., 2005	Albino Dunkin-Hartley guinea-pig	Contractility
Matsumoto et al., 2005a	ddY mice	Tolerance Cross-tolerance Withdrawal signs
Matsumoto et al., 2005b	Albino guinea pigs	Vas deferens contractility
Raja Aziddin et al., 2005	Sprague-Dawley rats	Inflammation
Kumarnsit et al., 2006	Wistar rats	Acute and chronic effects of MS extract Food and water intakes and weight gain
Matsumoto et al., 2006a	Albino guinea-pig ddY mice	Ileum contractility Vas deferens contractility Gastrointestinal transit
Matsumoto et al., 2006c	Mice (species not specified)	Tail-flick Hot plate Gastrointestinal transit
Matsumoto et al., 2006d	Mice (species not specified)	Tail-flick Hot plate
Kumarnsit et al., 2007a	Swiss albino mice	Ethanol withdrawal treatment
Kumarnsit et al., 2007b	Wistar rats	Force swimming

ddY-mice = Deutschland, Denken, and Yoken mice

Table 2.1, continued

Study	Animal(s)	Test(s)
Reanmongkol et al., 2007	Swiss mice	Pentobarbital-induced sleep
Chittrakarn et al., 2008	Wistar rats	Intestinal transit Body weight Cholecystokinin Assay
Moklas et al., 2008	Sprague Dawley rats	Locomotors activity
Mossadeq et al., 2009a	Balb C mice Sprague-Dawley rats	Writhing Formalin and Carrageenan-induced Paw Oedema
Mossadeq et al., 2009b	Guinea-pig (species not specified)	Contractility
Apryani et al., 2010	ICR mice	Object location task Open field
Cheeha et al., 2010	Wistar rats	EEG test on ethanol withdrawal rats
Chittrakarn et al., 2010	Wistar rats	Test on isolated phrenic nerve–hemidiaphragm, isolated hemidiaphragm and sciatic nerve preparation
Farah Idayu et al., 2010	ICR mice	Force Swimming Tail suspension Open field Corticosterone assay
Mossadeq et al., 2011	Guinea-pig (species not specified)	Ileum contractility
Nurul Akmaryanti et al., 2011	Bulb C mice	Force Swimming Tail suspension Locomotors activity

ICR mice = imprinting control region mice

Evaluation of the pharmacological effects of MS was difficult given the considerable variation among the studies in the designs, animal strains and preparations. Issues related to dose and treatment duration were not examined in this review because the focus of our review was to examine the pharmacological effects of MS. Given the lack of dose-response studies, it was unclear whether any of the studies have used an optimal dose.

We had intended to perform a meta-analysis however; we concluded that such an analysis was not appropriate given the variability in the research findings and no effect size was reported for any study. Thus, we presented the data descriptively.

Many of the animal studies on MS seem to support the effect reported in human such as analgesic effects, reduction of withdrawal symptoms and antidiarrheal effects (Suwanlert, 1975).

The main issue involving animal studies is the use of unknown optimal dose to examine the effects. Perhaps animal’s studies should start with a standardised dose and methods with some allowance of sample size that is statistically possible to yield better result. Even though the data are still preliminary, they are still useful to guide future studies.

2.3.3 Methodological quality of included studies

In an attempt to collect all available literature, we included trials of all methodological types. Out of 44 publications, 28 studies (64 %) were published in a peer reviewed journal. All studies used appropriate animal model and healthy animals (100%). None of the studies mentioned blinding of interventions and outcome treatment or control, blinding of outcome assessor and sample size calculation. Controls of temperature were reported by 28 publications (64%), compliance with animal welfare regulations by 21 publications (48%), and declaration for potential conflict of interest by 31 publications (70%). Random allocation to treatment or control was mentioned by three studies (7%), however they did not describe their methods of randomisation. The summary of the methodological qualities of the included studies are as shown in Table 2.2.

Table 2.2: The quality of included studies based on the checklist from CAMARADES

Author(s), year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Grewal, 1932a	√	-	-	-	-	√	√	-	-	-
Macko et al., 1972	√	-	-	-	-	-	√	-	-	-
Abdullah, 1985	-	√	-	-	-	-	√	-	-	√
Hashim, 1987	-	√	?	-	-	-	√	-	-	√
Looi, 1990	-	-	-	-	-	-	√	-	-	√
Dharmaraj, 1991	-	-	-	-	-	-	√	-	-	√
Inayat Hussain, 1991	-	-	-	-	-	-	√	-	-	√
Shahar et al., 1991	-	-	-	-	-	-	√	-	-	-
Ahmad, 1992	-	-	-	-	-	-	√	-	-	√
Aziz, 1993	-	√	?	-	-	-	√	-	-	√
Husain, 1993	-	-	-	-	-	-	√	-	-	√
Matsumoto et al., 1996a	√	√	-	-	-	-	√	-	√	√
Matsumoto et al., 1996b	√	√	-	-	-	-	√	-	√	√
Matsumoto et al., 1997	√	√	-	-	-	-	√	-	√	√

Studies fulfilling the criteria of: (1)peer reviewed publication, (2) control of temperature, (3) random allocation to treatment or control, (4) blinded to treatments, (5) blinded assessment of outcome, (6) use of anaesthetic without significant intrinsic neuroprotective activity, (7) appropriate animal model (aged, diabetic, hypertensive or healthy), (8) sample size calculation, (9) compliance with animal welfare regulations, and (10) statement of potential conflict of interest

? = unclear, √ = compliance to criteria, - = not available

Table 2.2 continued

Author(s), year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Watanabe et al., 1997	√	√	-	-	-	-	√	-	√	√
Abushwereb, 1998	-	√	?	-	-	-	√	-	-	√
Idid et al., 1998	-	√	-	-	-	-	√	-	-	-
Thongpradichote et al., 1998	√	√	-	-	-	-	√	-	√	-
Takayama et al., 2000	√	-	-	-	-	-	√	-	-	-
Latif et al., 2002	-	√	-	-	-	-	√	-	√	√
Takayama et al., 2002	√	√	-	-	-	-	√	-	-	√
Tsuchiya et al., 2002	√	√	-	-	-	-	√	-	-	-
Matsumoto et al., 2004	√	√	-	-	-	-	√	-	√	√
Raja Aziddin et al., 2005	√	√	-	-	-	-	√	-	-	-
Horie et al., 2005	√	√	-	-	-	-	√	-	√	√
Matsumoto et al., 2005a	√	√	-	-	-	-	√	-	√	√
Matsumoto et al., 2005b	√	√	-	-	-	-	√	-	√	√
Kumarnsit et al., 2006	√	√	-	-	-	-	√	-	√	√
Matsumoto et al., 2006a	√	√	-	-	-	-	√	-	√	√
Matsumoto et al., 2006c	√	-	-	-	-	-	√	-	-	-
Matsumoto et al., 2006d	√	-	-	-	-	-	√	-	-	-

Studies fulfilling the criteria of: (1) peer reviewed publication, (2) control of temperature, (3) random allocation to treatment or control, (4) blinded to treatments, (5) blinded assessment of outcome, (6) use of anaesthetic without significant intrinsic neuroprotective activity, (7) appropriate animal model (aged, diabetic, hypertensive or healthy), (8) sample size calculation, (9) compliance with animal welfare regulations, and (10) statement of potential conflict of interest

? = unclear, √ = compliance to criteria, - = not available

Table 2.2 continued

Author(s), year	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Reanmongkol et al., 2007	√	√	-	-	-	-	√	-	√	√
Kumarnsit et al., 2007a	√	√	-	-	-	-	√	-	√	√
Kumarnsit et al., 2007b	√	√	-	-	-	-	√	-	√	√
Chittrakarn et al., 2008	√	√	-	-	-	-	√	-	√	√
Moklas et al., 2008	√	-	-	-	-	-	√	-	√	√
Mossadeq et al., 2009a	√	√	-	-	-	-	√	-	√	√
Mossadeq et al., 2009b	-	-	-	-	-	-	√	-	-	√
Apryani et al., 2010	√	√	-	-	-	-	√	-	√	√
Cheeha et al., 2010	-	-	-	-	-	-	√	-	-	-
Chittrakarn et al., 2010	√	√	-	-	-	-	√	-	√	√
Farah Idayu et al., 2010	√	√	-	-	-	-	√	-	√	√
Mossadeq et al., 2011	-	-	-	-	-	-	√	-	-	-
Nurul Akmaryanti et al., 2011	-	-	-	-	-	-	√	-	-	-

Studies fulfilling the criteria of: (1)peer reviewed publication, (2)control of temperature, (3) random allocation to treatment or control, (4) blinded to treatments, (5) blinded assessment of outcome, (6) use of anaesthetic without significant intrinsic neuroprotective activity, (7) appropriate animal model (aged, diabetic, hypertensive or healthy), (8) sample size calculation, (9) compliance with animal welfare regulations, and (10) statement of potential conflict of interest

? = unclear, √ = compliance to criteria, - = not available

Overall there were few high-quality studies to make a firm conclusion on specific pharmacological effects reported. A major disadvantage of the evaluation instrument was that it gives more weight to quality of reporting than to actual methodological quality.

Measures to avoid bias were infrequently reported, while some authors might have taken such measures but they had not reported them. Studies that do not report these measures are much more likely to overstate the effects examined (Bebarta et al., 2003). Even though reducing the number of animals used is important, investigators often conducted studies that are too small to detect the anticipated effect.

As summary scores to identify high-quality trials are controversial (van der Worp et al., 2010), we have presented the absence and presence of an individual criterion to allow easier interpretation of studies quality.

Some guidelines have been proposed to improve the quality in reporting of animal studies to make it easier to conduct systematic review and meta-analysis (Kilkenny et al., 2010). Currently, animal experimentation requires more attention to reorganise standard procedures to assign animal groups to allow randomisation and blinding.

Keeping in mind the overall quality of evidence from a systematic review is highly dependent on the quality of its included studies; results should be interpreted with caution. Most studies thus far are of small sample sizes using non-oral route to administer MS in animals and monitor outcomes over only a short follow-up period, limiting generalising results and extrapolation of findings to human.

2.3.4 The Pharmacological properties of MS

We identified 44 studies examining a variety of pharmacological activities of MS as follows:

(a) Antinociceptive activity

Table 2.3a shows antinociceptive activities of MS were investigated in 20 animal studies which used mainly tail-flick, hot-plate and tail-pinch test. Various controls were used such as morphine, paracetamol, and codeine. The most commonly used control was morphine (17 studies). One study used rabbit (Grewal, 1932a) while all other studies used either mice or rat from difference species.

The MS constituents used were mainly mitragynine (9 studies); 7-hydroxymitragynine (5 studies) and MS extract (7 studies). All studies use latency for pain except Grewal (1932a) that examined anaesthetic effect. The majority of the studies investigated MS effects at opioid receptor subtypes (Hashim, 1987; Matsumoto et al., 1996a; Thongpradichote et al., 1998; Matsumoto et al., 2005a; Reanmongkol et al., 2007; Mossadeq et al., 2009a) and involvement of noradrenergic (Ahmad, 1992; Husain, 1993; Matsumoto et al., 1996a) and serotonergic systems (Matsumoto et al., 1996a).

The hot plate and tail flick tests are commonly used to investigate the involvement of supraspinal (Yaksh and Rudy, 1976) and spinal component (Mayer and Liebeskind, 1974). The antinociceptive activity of MS probably involves several systems including the supraspinal system (Matsumoto et al., 1996b), the stimulation of postsynaptic α_2-adrenoceptors (Ahmad, 1992; Matsumoto et al., 1996a) as well as modulation of 5-HT_{2A} receptors (Matsumoto et al., 1996a).

The analgesic action of MS has been shown to be blocked by naloxone, a pure opioid antagonist (Gutstein and Akil, 2001) which suggest the involvement of opioid receptors in antinociceptive activity. Several studies supported the stimulation of opioid receptors by mitragynine and 7-hydroxymitragynine (Matsumoto et al., 1996b; Thongpradichote et al., 1998; Matsumoto et al., 2004). The two compounds are structurally associated to Yohimbine (Babu et al., 2008) but they act primarily at μ-opioid receptor (Thongpradichote et al., 1998; Takayama et al., 2002; Matsumoto et al., 2004; Matsumoto et al., 2006a,d). However, they have less affinity on δ and κ-opioid receptor (Thongpradichote et al., 1998). All studies involving mitragynine show that mitragynine has less potency compared to morphine (Hashim, 1987; Dharmaraj, 1991; Inayat Hussain, 1991) and codeine (Macko et al., 1972), while 7-hydroxymitragynine has been shown to be more potent than morphine (Matsumoto et al., 2004; Matsumoto et al., 2005a; Matsumoto et al., 2006a,c) as it acts predominantly on μ1 and μ2-opioid receptor (Matsumoto et al., 2004; Matsumoto et al., 2006a). The opioid effect seems to explain the human use of MS as a folk remedy for various illnesses including pain and opiate withdrawal management (Vicknasingam et al., 2010).

Table 2.3 (a): Pharmacological properties of *Mitragyna speciosa*: Antinociceptive effects

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Grewal, 1932a	Rabbit (strain not specified)	*Local anaesthetic test* Mitragynine 0.01-1% topical application	No control	Duration of complete anaesthesia	Dose of mitragynine 0.01% reached complete anaesthesia at 7 minutes which was faster compared to higher dose (0.1% reached at 30 minutes and 1% reached at 42 minutes)
Macko et al., 1972	Rat (strain not specified)	*D'Amour and Smith and Randall and Selitto test* Mitragynine (dose not specified) p.o.; Mitragynine 31mg/kg s.c.	Codeine d-propoxyphene	Latency for pain	Mitragynine was less potent compared to codeine either orally or subcutaneously.
	Dog (strain not specified)	*Hardy, Wolff and Goodel test* Mitragynine 2-24mg/kg p.o.	Codeine d-propoxyphene	Latency for pain	Mitragynine was less potent than control.

p.o. = oral, s.c. = subcutaneous

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
	Mice (strain not specified)	*Hot plate test* Mitragynine 92mg/kg p.o and s.c.	Codeine	Latency for pain	Mitragynine showed more potent effect compared to codeine either orally or subcutaneously.
Hashim, 1987	Swiss Webster mice, Wistar rats	*Hot-plate, Tail-flick* MS chloroform extract 3.75-125 mg/kg i.p. and 0.5-8 µg/kg i.c.v.; MS petroleum ether extracts 12.5-200mg/kg i.p.	Morphine Naloxone	Latency for pain	Antinociception increased with extract dose but has lower potency to morphine. Naloxone showed antagonism to MS.
Dharmaraj, 1991	Mice *(Mus Musculus)*	*Tail-flick test* MS chloroform extract 100-300 mg/kg i.p.	Morphine Olive oil Saline	Latency for pain	Antinociceptive effect of MS was dose dependent. MS extract 250mg/kg was equipotent to morphine 4.6 mg/kg.

i.c.v. = intracerebroventricular, i.p. = intraperitoneal, p.o. = oral, s.c. = subcutaneous

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Inayat Hussain, 1991	Mice *(Mus Musculus)*	*Tail-flick test* MS chloroform extract 100-300 mg/kg i.p.	Morphine	Latency for pain	MS extract 262.7mg/kg was equipotent to morphine 4.8 mg/kg Analgesic index was 0.5.
Shahar, 1991	Mice *(Mus Musculus)*	*Tail-flick test* MS crude extract; Mitragynine (route of administration not specified)	Morphine	Latency for pain	Analgesic effect was clearly observed from mitragynine but the effect was not as potent as morphine. Crude extracts did not show any significant analgesic effect.
Ahmad, 1992	Rat (species not specified)	*Tail-flick test* MS extract 100mg/kg i.p.	Clonidine Morphine Yohimbine	Latency for pain	Clonidine did not show additive effect to the analgesic effect of MS and morphine. Yohimbine was found to block the analgesic effect of MS and morphine.

i.p. = intraperitoneal

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Husain, 1993	Albino mice	*Tail-flick test* Mitragynine 100mg/kg i.p.	Morphine Yohimbine	Latency for pain	Mitragynine and morphine induced antinociception. Yohimbine antagonised analgesic effects of mitragynine and morphine.
Matsumoto et al., 1996a	ddY-mice	*Hot-plate and tail-pinch test* Mitragynine 1-10µg i.c.v.	6-OHDA Cyproheptadine Idazoxan Morphine p-CPA Reserpine Vehicle	Latency for pain	Mitragynine was concluded to be acting at supraspinal level. However, α-adrenoceptor antagonist inhibited the action of mitragynine. Descending noradrenergic and serotonergic systems were involved in the antinociceptive activity of mitragynine.

i.p. = intraperitoneal, i.c.v. = intracerebroventricular, 6-OHDA = 6-hydroxydopamine, p-CPA = para-cholorophenalanine, ddY-mice = Deutschland, Denken, and Yoken mice

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Matsumoto et al., 1996b	ddY-mice	*Hot-plate and tail-pinch test* Mitragynine 5- 30 mg/kg i.p.; Mitragynine 1-10µg/ mouse i.c.v.	Naloxone Saline Vehicle	Latency for pain	Both i.p and i.c.v. mitragynine induced antinociception which were reversed by naloxone. Supraspinal opioid system was at least partly involved in antinociception by mitragynine.
Idid et al., 1998	Albino Mice	*Hot tail-flick and cold tail-flick test* Mitragynine 200 mg/kg p.o.	Morphine Paracetamol Saline	Latency for pain	Mitragynine showed reduction in writhing response and increased in analgesia.

i.p.=intraperitoneal , i.c.v.= intracerebroventricular, p.o.= oral, ddY-mice = Deutschland, Denken, and Yoken mice

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Thongpradichote et al., 1998	ddY-mice	*Tail-pinch and hot plate test* Mitragynine 10 μg i.c.v.	Cyprodime Morphine Naloxonazine Naltrindole U50488H Vehicle	Latency for pain	Mitragynine was capable of directly interacting with supraspinal μ or δ receptor subtypes to produce antinociceptive effect on mechanical and thermal-stimuli. Activity of mitragynine on κ-opioid receptors might be indirect thus required further evaluation.
Takayama et al., 2002	Mice (species not specified)	*Tail-flick test* Mitragynine i.c.v (dose not specified)	Mitragynine pseudoindoxyl Morphine Naloxone	Latency for pain	Analgesic effects of mitragynine, mitragynine pseudoindoxyl and morphine was dose-dependent and antagonized by naloxone.
Matsumoto et al., 2004	ddY Mice	*Tail-flick test and hot-plate test* 7-hydroxymitragynine 2.5-20mg/kg s.c., p.o.	Morphine	Latency for pain	7-hydroxymitragynine possessed more potent antinociceptive action after s.c. or p.o. administration compared to morphine.

ddY-mice = Deutschland, Denken, and Yoken mice, i.c.v. = intracerebroventricular, s.c. = subcutaneous, p.o. = oral

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Matsumoto et al., 2005	ddY mice	*Tail-flick test* 7-hydroxymitragynine 2.5-10mg/kg s.c.	Cyprodime Morphine Naloxone Naltrindole NorBNI Vehicle	Latency for pain	7-hydroxymitragynine induced antinociception through activation of μ-opioid receptor.
Matsumoto et al., 2006a	ddY mice	*Tail-flick and hot plate test* 7-hydroxymitragynine 2 mg/kg s.c	Morphine Naloxonazine Naloxone Naltrindol NorBNI Vehicle β-FNA	Latency for pain	7-hydroxymitragynine was more potent than morphine

ddY-mice = Deutschland, Denken, and Yoken mice, s.c. = subcutaneous, NorBNI = Norbinaltorphimine, β-FNA = β-funaltrexamine

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Matsumoto et al., 2006c	Mice	*Tail-flick and hot plate test* 7-hydroxymitragynine 0.5-2 mg/kg s.c.; 7-hydroxymitragynine 2-8 mg/kg p.o.	Morphine	Latency for pain	7-hydroxymitragynine was more potent than morphine.
Matsumoto et al., 2006d	Mice (species not specified)	*Tail-flick test and hot plate test* 7-hydroxymitragynine (dose and administration not specified)	Morphine β-FNA	Latency for pain	7-hydroxymitragynine antinociception was 5-fold more potent than morphine and this effect was antagonised by β-FNA.

p.o. = oral, s.c. = subcutaneous, β-FNA = β-funaltrexamine

Table 2.3 (a), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Reanmongkol et al., 2007	Swiss mice	*Tail-flick and hot-plate test* MS extract (methanol) 50-200 mg/kg p.o. MS extract (alkaloid) 5-20 mg/kg p.o.	Morphine Naloxone Vehicle	Latency for pain	Both methanol and alkaloid extract of MS produced antinociception that were blocked by naloxone.
Mossadeq et al., 2009a	Bulb C mice	*Hot plate test* MS extract (methanol) 50-200 mg/kg i.p.	Acetylsalicylic acid Morphine Naloxone Saline	Latency for pain	MS methanol extract reduced nociception on mice in hot plate test but antagonised by naloxone.

i.p. = intraperitoneal, p.o. = oral

(b) Effects on biogenic amines level

Five studies examined the effect of MS on biogenic amines levels (Table 2.3b). Abushwereb (1998) and Latif et al. (2001) found that biogenic amine levels (noradrenalin, serotonin and dopamine) were significantly altered at various parts of the brains of treated rats, while three studies (Dharmaraj, 1991; Inayat Hussain, 1991; Husain, 1993) found there were no significant effects on biogenic amines levels.

Some of the changes of these biogenic amines could possibly explain the changes of behaviour on mice in antidepressant test (Kumarnsit et al., 2007b). However, data are still lacking to make a firm conclusion on the effects of MS on biogenic amines levels.

Table 2.3 (b): Pharmacological properties of *Mitragyna speciosa*: Effect of biogenic amines level

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Dharmaraj, 1991	Mice *(Mus Musculus)*	*Serotonin Assay* MS chloroform extract 100-300 mg/kg i.p.	Morphine Olive oil Saline	Serotonin level	MS extract did not affect the serotonin level during analgesia.
Inayat Hussain, 1991	Mice *(Mus Musculus)*	*Noradrenalin Assay* MS chloroform extract 100-300 mg/kg i.p.	Morphine	Noradrenalin level	No significant difference was seen in the level of noradrenalin in mice brain during analgesia between MS and morphine group.
Husain, 1993	Albino mice	*Noradrenalin Assay* Mitragynine 100mg/kg i.p.	Morphine Yohimbine	Noradrenalin level	No significant changes on the level of noradrenalin in hypothalamus and brain stem area during analgesia of both mitragynine and morphine treated mice.

i.p. = intraperitoneal

Table 2.3 (b), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Abushwereb, 1998	Wistar rats	*Biogenic Amines Assay* MS methanolic extract 50-400 mg/kg p.o.	Diazepam	Biogenic amines levels	Test of MS extract and diazepam on rats showed: 1. Serotonin levels were found to be higher in substantia nigra. Serotonin metabolite, 5-hydroxyindoleacetic acids were found higher in raphe nuclei and nucleus accumbens. 2. Increase noradrenalin levels in locus coeruleus, nucleus accumbens, and substantia niagra and raphe nuclei. 3. Dopamine levels were increased in substantia niagra and nucleus accumbens.

p.o. =oral

Table 2.3 (b), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Latif et al, 2001	Sprague Dawley rats	*Biogenic Amines Assay* Mitragynine 0.1 and 1.0 mg/rat i.p.	Morphine Saline	Biogenic amines levels	Test of mitragynine and morphine on rats showed: 1. Noradrenalin and serotonin levels depleted in hypothalamus, frontal cortex and brain stem at high dose. 2. Dopamine increased in frontal cortex and brain stem at high dose. 3. No changes of noradrenalin, serotonin and dopamine in hypothalamus, frontal cortex and brain stem at low dose.

i.p.= intraperitoneal

(c) Behavioural and cognitive functions

Table 2.3c shows 11 studies assessed behavioural and cognitive functions. A variety of tests were used including force swimming test, open field test, maze test and tail-suspension test. All these tests observed latency of immobility and locomotors activities. Two studies examined cognitive function, one on memory (Apryani et al., 2010) and one on drug discriminative properties and self administration (Aziz, 1993).

All nine studies which examined the antidepressive effects showed MS extract or mitragynine have significant effects on depression. Only Farah Idayu et al. (2011), showed the involvement of cortisone in antidepressive effect caused by MS.

Locomotors test showed that there were no significant effects of either MS extract or mitragynine on locomotors activity except for three studies (Abdullah, 1985; Moklas et al., 2008; Apryani et al., 2010). Abdullah (1985) found that there was an increase in motor activity at lower dose while Apryani et al. (2010) and Moklas et al. (1985) found at any dose locomotors activity was significantly reduced.

Cognitive and behaviour studies done by Aziz (1993) reported that MS cause addiction to rats however found that MS do not have similar discriminative cues like other substance of abuse (amphetamine, cocaine pentobarbitone and morphine). Another cognitive study (Apryani et al., 2010) showed MS caused impairment in memory.

Table 2.3 (c): Pharmacological properties of *Mitragyna speciosa*: Behaviour and cognitive functions

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Abdullah, 1985	Mice (species not specified)	*Co-ordination test (incline plane)* MS chloroform extract 1-256 mg/kg i.p.	Morphine	Locomotors activity	MS extract increased motor activity at lower doses but at higher doses produced depressant effects.
Aziz, 1993	Wistar rat	*Drug Discrimination Test* MS methanolic extract 9.38-150 mg/kg; Mitragynine 2.5-20 mg/kg	Cocaine d-amphetamine Haloperidol Morphine Naloxone Pentobarbital Quinine Saline	Drug preference by rats.	MS was able to cause self-administration in rats. MS was found to produce weak discriminative cues and did not substitute any of the tested drugs cues.

i.p.= intraperitoneal

Table 2.3 (c), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Matsumoto et al., 1996 b	ddY-mice	*5-Methoxy-N,N-dimethyltryptamine-induced head twitch and spontaneous motor activity test* Mitragynine 1-30 mg/kg i.p.	6-OHDA Idazoxan Nomifensine p-CPA Reserpine Ritanserin Saline Yohimbine	Number of head twitch response Number of spontaneous motor activity	α_2-adrenoceptor blocker attenuated mitragynine suppression on the head twitch response suggesting stimulation of postsynaptic α_2-adrenoceptor or blockade of 5-HT_{2A} receptor or both. Mitragynine did not significantly change spontaneous motor activity.
Abushwereb, 1998	Albino mice Wistar rats	*Force Swimming test, open field test and plus-maze test* MS methanolic extract 50-400 mg/kg p.o.	Imipramine Distilled water Yohimbine Diazepam	Latency of immobility Spontaneous motor activity	MS reduced immobility time in force swimming test. No significant changes in locomotor activity.

ddY-mice = Deutschland, Denken, and Yoken mice, i.p.=intraperitoneal, p.o.=oral, p-CPA=p-chlorophenlalanine, 6-OHDA=6-hydroxydopamine, 5-HT_{2A} = 5-Hydroxytryptamine 2A

Table 2.3 (c), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
				Latency of exploration, number of line crossed and number of entries of the maze arm.	Increased time spent at the open arm, number of line crossed and number of entries, thus MS showed anxiolytic-like effects in plus-maze test.
Kumarnsit et al., 2006	Wistar rats	*Test on acute and chronic effects of MS extract administration on food and water intakes and weight gain* *Acute*			
		MS extract 15-50 mg/kg i.p.	Imipramine Saline	Weight of food and water consumption Body weight	MS extract and Imipramine reduced food and water intake in rats and slowed down weight gained. However Imipramine was more potent.

i.p.=intraperitoneal

Table 2.3 (c), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
		Chronic			
		MS extract 40 mg/kg i.p. for 60days	Saline	Weight of food and water consumption Body weight	Chronic administration suppressed food and water intake and slowed weight gained in rats.
Kumarnsit et al., 2007a	Wistar rats	*Force swimming test*	Saline	Latency of immobility	The time to immobility of rats in force swimming test was reduced by MS extract.
		MS extract 60 or 90mg/kg i.p.			
Kumarnsit et al., 2007b	Swiss albino mice	*Tail-suspension and spontaneous motor activity test*	Vehicle	Latency of immobility	Time to immobility was reduced by MS extract.
		MS extract (aqueous) 100-500 mg/kg i.g.		Locomotors activity	MS have no effects on spontaneous motor activity.

i.g.= intragastric, i.p.= intraperitoneal

Table 2.3 (c), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Moklas et al, 2008	Sprague Dawley rats	*Locomotors box test* Mitragynine 1.0, 5.0. 10.0 and 30.0 mg/kg, i.p.	Amphetamine Saline Tween 80	Locomotors activity	Mitragynine reduced locomotors activity.
Apryani et al., 2010	ICR mice	*Object location task and open field test* Mitragynine 5-15 mg/kg i.p.	Amphetamine Saline Scopolamine Tween 80	Latency of exploration and locomotors activity	Mitragynine reduced locomotor activity and impaired performance on object location task thus, showed alteration on cognitive and behavioural function
Farah Idayu et al., 2010	ICR mice	*Force Swimming test, tail suspension test and open field test and corticosterone assay* Mitragynine 5-30 mg/kg i.p.	Amitriptyline Amphetamine Fluoxetine Vehicle	Latency of immobility	Mitragynine prolonged the immobility of mice in force swimming test and tail suspension test.
				Locomotors activity	Mitragynine produced no significant locomotors effects.

i.p.= intraperitoneal, ICR mice = Imprinting control region mice

Table 2.3 (c), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
				Serum corticosterone	Mitragynine and antidepressant drug reduced corticosterone levels.
Nurul Akmaryanti et al, 2011	Bulb C mice	*Force Swimming test, tail suspension test, Locomotors activity test*	Amitriptyline Vehicle	Latency of immobility	MS reduced the immobility time of mice in both force swimming test and tail suspension test.
		MS chloroform extract 10, 30,100 mg/kg		Locomotors activity	No significant effect on locomotor activity of mice.

It is possible that the antidepressive effect of MS might be mediated through noradrenergic and serotonergic system by stimulating the release of noradrenalin and serotonin from nerve terminals of descending monoaminergic neuron (Matsumoto et al., 1996a). The blockade of these monoamines uptake by MS could have increased the extracellular levels of serotonin and noradrenalin, which then improved symptoms of depression (Kumarnsit et al., 2007b). It was found that the dorsal raphe nucleus plays a crucial role in the effect of an antidepressant.

The purpose of locomotor test by using open field test is to remove false positive result in fast swimming test and tail-suspension test due to hyperkinesias (Bourin et al., 2001; Butterweck et al., 2003; Kwon et al., 2010), thus the test minimises the possibility of psycho-stimulant effects and therefore confirmed the antidepressant effects (Borsini and Meli, 1988). Mitragynine shows no psycho-stimulant activity in the brain. In addition, the finding suggested that reduction of immobility time elicited by mitragynine in fast swimming test as well as in tail suspension test specifically arises via its antidepressant mechanism. MS produced no significance effect over locomotors activity and on pentobarbital-induced sleep in mice (Reanmongkol et al., 2007). Locomotor activity was reduced probably due to the sedative factor (Moklas et al., 2008) as reported in earlier studies (Reanmongkol et al., 2007).

Since very limited studies are available to explain the cognitive changes shown by MS, it is difficult to make a firm conclusion on the behavioural effects of MS

(d) Peripheral nervous systems

Table 2.3d shows that only two studies investigated the effects of MS on peripheral nervous systems (Grewal, 1932a; Chittrakarn et al., 2010). The types of nerves and parameters being investigated were very different between the two studies. Both studies found that mitragynine did not produce any statistically significant effect on nerves.

Table 2.3 (d): Pharmacological properties of *Mitragyna speciosa*: Effects on peripheral nerves

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Grewal, 1932a		**Peripheral nerves test*			
	Frog (strain not specified)	Mitragynine dilution 1:1000	No control	Nerve conduction	No effect.
	Cat (strain not specified)	Mitragynine (no dose) painted on sciatic nerve	No control	Blood pressure	Blood pressure did not drop even after faradic stimulation of sciatic nerve.
Chittrakarn et al., 2010	Wistar rats	**Test on isolated phrenic nerve–hemidiaphragm, isolated hemidiaphragm and sciatic nerve* MS extract (methanol) 0.1-1mitragynine/ml Mitragynine 0.0156 mg/ml	D-tubocurarine Neostigmine Pancuronium Sodium pentobarbital Succinylcholine Tetraethylammonium	Nerve conduction, amplitude and duration.	MS extract and mitragynine decreased muscle twitch on isolated phrenic nerve-hemi diaphragm and decreased nerve conduction, amplitude and duration on action potential of isolated sciatic nerve.

*test using organ bath

(e) Withdrawal effects, dependence, tolerance and cross-tolerance

Table 2.3e shows six animal studies examined the behavioural changes and withdrawal signs of MS except for one study which assessed the brain waves. Two studies investigated MS effect on ethanol withdrawal (Kumarnsit et al., 2007a; Cheaha et al., 2010), three studies examined repeated 7-hydroxymitragynine dose on tolerance and cross-tolerance (Matsumoto et al., 2005a; Matsumoto et al., 2006c,d) while the two remaining studies assessed MS ability to stop opiate withdrawal response. Three studies used MS extract, one study (Macko et al., 1972) used mitragynine and three studies use 7-hydroxymitragynine (Matsumoto et al., 2005a; Matsumoto et al., 2006c,d).

Macko et al. (1972) and Hashim (1987) found that mitragynine did not suppress opiate withdrawal response.

MS does not seem to reduce withdrawal response due to opiates as claimed. The findings on its ability to reduce ethanol withdrawal response in mice are too preliminary to make any conclusion. It is interesting to note that after repeated dose of 7-hydroxymitragynine on mice, tolerance and cross-tolerance effects appeared to be similar to that of morphine (Matsumoto et al., 2005a).

DuPen et al. (2007) postulated that opioid tolerance was due to changes in opioid receptors by desensitization of opioid receptors after prolong exposure to opioid agonist and down-regulation of the receptor due to internalisation of the opioid receptor from the cell membrane.

Table 2.3 (e): Pharmacological properties of *Mitragyna speciosa*: Withdrawal effects, dependence, tolerance and cross-tolerance

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Macko et al., 1972		*Nalorphine antagonism tests*			
	Cat (strain not specified) Rat (strain not specified)	Mitragynine 18.4-46mg/kg i.p.	Codeine Morphine Nalorphine	Withdrawal signs	No effect by nalorphine to antagonise the effects of mitragynine in cats and rats.
Hashim, 1987	Wistar rats	*Dependence test* MS methanolic extract 2.025 g/kg i.p.	Morphine Saline	Behavioural changes	The rats were seen to be sedated, immobile, and non-aggressive and showed absence of exploratory behaviour. They showed piloerection, ptosis and hypersensitivity to noise. Symptoms were suppressed with morphine.

i.p.= intraperitoneal

Table 2.3 (e), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Matsumoto et al., 2005a	ddY mice	*Tolerance, cross-tolerance and withdrawal signs test.* 7-hydroxymitragynine 5-10mg/kg s.c.	Morphine Vehicle	Withdrawal signs	Repeated exposure to 7-hydroxymitragynine lead to tolerance, cross-tolerance and dependence similar to that seen with morphine.
Matsumoto et al., 2006c	Mice (strain not specified)	*Tolerance and cross-tolerance test* 7-hydroxymitragynine 0.5-2 mg/kg s.c.; 2-8 mg/kg p.o.	Morphine	Withdrawal signs	7-hydroxymitragynine has tolerance and cross-tolerance effect similar to morphine.
Matsumoto et al., 2006d	Mice (strain not specified)	*Tolerance and cross-tolerance test* 7-hydroxymitragynine (dose and administration not specified)	Morphine	Withdrawal signs	7-hydroxymitragynine has tolerance and cross-tolerance effect similar to morphine.

ddY-mice = Deutschland, Denken, and Yoken mice, p.o.= oral, s.c.= subcutaneous

Table 2.3 (e), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Kumarnsit et al., 2007a	Swiss albino mice	*Ethanol withdrawal treatment test* MS extract (aqueous) 100-500 mg/kg i.g	Vehicle Saline	Withdrawal sign	MS extract reduce ethanol withdrawal effect.
Cheaha et al., 2010	Wistar rats	*EEG test on ethanol withdrawal rats* MS extract 60 mg/kg (route of administration not specified)	No control	EEG changes	MS extract reversed the ethanol withdrawal induced change in EEG power from delta to beta wave.

i.g.= intragastric

(f) Sleep

Two studies assessed the effects of MS on duration of sleep (Looi, 1990; Reanmongkol et al., 2007). As shown in Table 2.3f both studies used MS extract and pentobarbitone as control in mice. Looi (1990) used MS chloroform extract given by intraperitoneal route while Reanmongkol et al. (2007) used methanol extract given orally.

Reanmongkol et al. (2007) found that there was no significant effect of MS on duration of sleep while Looi (1990) showed MS significantly reduced sleep time. The contradicting effect reported is difficult to explain. It is probably due to the different dose and route of administrations used in the two studies.

The effect reported in human is also contradictory; MS was reported to be used to overcome sleepiness, while some users reported trouble with sleeping associated with MS use (Assanangkornchai et al., 2007). More research is required in order to understand the effects of MS on sleep.

Table 2.3 (f): Pharmacological properties of *Mitragyna speciosa*: Sleep

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Looi, 1990	Mice *(Mus Musculus)*	*Sleep-time Induced by Pentobarbitone Test* MS crude extracts 60 mg/kg i.p. MS ethanol and chloroform extract 40 mg/kg i.p.	Olive oil Saline Pentobarbitone	Duration of sleep	Acute treatment with all MS extract decreased sleep time between 52% to 81% while sub acute treatment reduced sleep time between 83% to 89%.
Reanmongkol et al., 2007	Swiss mice	*Pentobarbital-induced sleep test* MS extract (methanol) 50-200 mg/kg p.o. MS extract (alkaloid) 5-20 mg/kg p.o.	Pentobarbitone Vehicle	Duration of sleep	Both extract did not significantly induce sedative effect.

i.p.= intraperitoneal, p.o.= oral

(g) Effects on gastrointestinal contractility

Table 2.3g shows ten studies studied MS effects on gastrointestinal contractility and three studies on motility or intestinal transit time. One study also explored the effects of MS on cholecystokinin level (Chittrakarn et al., 2008) and one study on opioid receptor-like 1 (ORL1) receptor (Horie et al., 2005).

All studies showed MS compounds and extracts reduced gastrointestinal contractility and motility. However, one study (Mossadeq et al., 2009b) found that MS has spasmogenic effect and that MS induced spasmogenic effect at lower dose and as the dose increased the spasmogenic effect is reduced in a concentration dependent manner.

A study on cholecystokinin (CCK) and opioid ORL1 was conducted but showed no significant effect on contractility with MS compounds. The involvement of CCK was due to the effects that, regulate intestinal motility and suppressed food intake (Moran and McHugh, 1982) while the involvement of ORL1 was studied due to similar sequence homology to opioid receptors that contributes to on pain perception and also gastrointestinal motility (Calo et al., 2000).

Table 2.3 (g): Pharmacological properties of *Mitragyna speciosa*: Effects on gastrointestinal

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Grewal, 1932a	Cat (strain not specified)	**Contractility test* Intestine (area not specified) Mitragynine dilution 1:20000, 1:100,000	Atropine Ringer's solution	Contractility	Mitragynine caused stimulation on the intestine by activating the vagus nerve endings.
Macko et al., 1972	Rat (strain not specified)	*Charcoal meal test* Mitragynine 36.8 mg/kg i.p, 55.2 mg/kg p.o.	Codeine Morphine	Motility inhibition	Mitragynine inhibited less gastrointestinal motility compared to control.
Shahar, 1991	Guinea pig (*Cavia porcerlus*)	**Ileum contractility test* MS crude extract Mitragynine (dose not specified)	Not specified	Contractility	Mitragynine inhibited ileum contractility.

*test using organ bath, i.p.= intraperitoneal, p.o.= oral

Table 2.3 (g), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Watanabe et al., 1997	Albino Dunkin-Hartley guinea-pig	**Ileum contractility test* Mitragynine 1 nM-25 μM	Atropine Hexamethonium Morphine Naloxone Nicardipine Tetrodotoxin Yohimbine	Contractility	Mitragynine inhibited ileum contractility via opioid receptor. Mitragynine did not show any effect on the smooth muscle contraction induced by acetylcholine and histamine. Mitragynine showed inhibitory effect on naloxone-precipitated withdrawal response of morphine.
Takayama et al., 2000	Guinea-pig ileum (strain not specified)	**Ileum contractility test* Mitragynine (dose not specified)	Mitragynine pseudoindoxyl Morphine	Contractility	Mitragynine was less potent than mitragynine pseudoindoxyl and morphine for the inhibition of the ileum. The methoxyl group in mitragynine at C-9 was responsible for the action at opioid receptor.

*test using organ bath

Table 2.3 (g), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Takayama et al., 2002	Albino Dunkin-Hartley guinea-pig	**Ileum contractility test* Mitragynine 7-hydroxymitragynine (dose not specified)	Morphine	Contractility	7-hydroxymitragynine has higher contractility inhibition potencies than morphine.
Matsumoto et al., 2004	Albino Dunkin-Hartley guinea-pig	** Ileum contractility test* 7-hydroxymitragynine 300 nM Mitragynine (dose not specified)	Morphine Vehicle	Contractility	7-hydroxymitragynine inhibited contractility better than morphine and mitragynine.

*test using organ bath

Table 2.3 (g), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Horie et al., 2005	Albino Dunkin-Hartley guinea-pig	**Ileum contractility test* Mitragynine 3 µM Crude MS extract 1-300 µg/ml Crude extract base (n-BuOH, AcOH-insoluble and water soluble fractions) 10-300 µg/ml Speciogynine 30µM Paynantheine 30µM Speciocilitine 30µM	Morphine Verapamil Naloxone Vehicle	Contractility	7-hydroxymitragynine was more potent than morphine and mitragynine. It acts on opioid receptor but not on ORL1 receptor.
		**Test on ORL1 receptor* 7-hydroxymitragynine 100 nM Speciociliatine 30 µM	Morphine Nociceptin		

*test using organ bath, AcOH = acetic acid, n-BuOH = n-butanol, ORL1 = opioid receptor like 1

Table 2.3 (g), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Matsumoto et al., 2006a	Albino guinea-pig	*_Ileum contractility_ 7-hydroxymitragynine 100 nM	Cyprodime DAMGO norBNI U69593	Contractility	Contractility was inhibited by 7-hydroxymitragynine which acts predominantly on μ-opioid receptors, especially on μ1-opioid receptors
	ddY mice	*Gastrointestinal transit test* 7-hydroxymitragynine 0.25-4 mg/kg s.c	Morphine Naloxone Naltrindol Vehicle β-FNA	Gastrointestinal transit time and relative potency	For gastrointestinal transit, 7-hydroxymitragynine has about 4.9–6.4 times less constipating effect than morphine at equivalent antinociceptive doses.
Matsumoto et al., 2006c	Mice (strain not specified)	*GI transit test* 7-hydroxymitragynine 1-4 mg/kg p.o, s.c.	Morphine	Gastrointestinal transit time	7-hydroxymitragynine is more potent and has less constipating effect than morphine.

*test using organ bath, ddY-mice = Deutschland, Denken, and Yoken mice, p.o. = oral, s.c. = subcutaneous, DAMGO = D-Ala^2, N-MePhe^4 Gly-ol^5]-enkephalin, norBNI = norbinaltorphimine, U69593 = (5α,7α,8β)-(+)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide, β-FNA = β-funaltrexamine

Table 2.3 (g), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Chittrakarn et al., 2008	Wistar Rats	*Test on intestinal transit, effect on body weight and CCK assay.* MS extract (methanol) 50 -400 mg/kg p.o.	Loperamide Morphine Vehicle	Intestinal transit	MS extract has antidiarrheal effect by decreasing intestinal transit time.
				Body weight	The effect on decrease in body weight was similar to morphine.
				CCK level	Plasma CCK was not affected by the extract.
Mossadeq et al., 2009a	Guinea-pig (strain not specified)	**Ileum contractility test* MS extract (methanol) 0.01-5.00 mg/ml	No control	Contractility	At lower dose, MS extract caused stimulatory effect on the ileum. At higher dose it produced significant concentration-dependent inhibition.

*test using organ bath, CCK=cholecystokinin, p.o. = oral

Table 2.3 (g), continued

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Mossadeq et al, 2011	Guinea pig (strain not specified)	**Ileum contractility test* MS methanolic extract 0.1-5 mg/ml	Histamine Serotonin Bradykinin Prostaglandin E2	Contractility	At lower dose MS produced spasmogenic effect but at higher dose inhibited contractility.

*test using organ bath

MS has been reported to inhibit contractility of guinea-pig ileum through the opioid receptor as well as α_2-adrenoceptor (Watanabe et al., 1997). On ileum contractility mitragynine was reported to be 10-fold less potent than morphine (Watanabe et al., 1997), however 7-hydroxymitragynine is more potent than morphine and other MS compounds (Takayama et al., 2002; Matsumoto et al., 2004; Horie et al., 2005; Matsumoto et al., 2006a,c). Probably all MS compounds have less constipating effect compared to morphine due to less inhibition of gastrointestinal motility at analgesic doses (Matsumoto et al., 2006a,c). MS also showed reduction of intestinal transit and exert antidiarrheal properties (Chittrakarn et al., 2008).

Spasmogenic effect of MS at low doses is probably induced by histamine, serotonin, bradykinin and prostaglandin E2 which decreased as the concentration of MS increased indicating the effect is blocked in a concentration-dependent manner (Mossadeq et al., 2009b). The spasmogenic effects seen in animal studies could also explain the use of MS in human in small doses to treat diarrhoea.

(h) Effects on vas deferens smooth muscle

Three studies examined the effects on contractility of vas deferens (Table 2.3h) of guinea-pig and rat. The compounds tested were MS extract, mitragynine and 7-hydroxymitragynine. All studies showed inhibition of vas deferens contractility.

Table 2.3 (h): Pharmacological properties of *Mitragyna speciosa*: Effects on vas deferens

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Shahar, 1991	Rat (*Rattus norvegicus*)	**Vas Deferens contractility test* MS crude extract Mitragynine	Not specified	Contractility	Vas deferens contractility was reduced.
Matsumoto et al., 2005b	Albino guinea-pig	**Vas deferans contractility test* Mitragynine 0.01 –30 µM	Hexamethonium Morphine Prazosin Tetrodotoxin α,β-methylene ATP	Contractility	Mitragynine inhibited vas deferens contraction elicited by nerve stimulation, probably through Ca^{2+} channels.
Matsumoto et al., 2006a	Albino guinea-pig ddY mice	**Vas deferens contractility test* 7-hydroxymitragynine 300nM	DAMGO DPDPE Naltrindol U69593	Contractility	Contractility was inhibited by 7-hydroxymitragynine, through the µ-opioid receptors.

*test using organ bath, ATP = adenosine triphosphate, DAMGO = D-Ala^2, N-MePhe^4, Gly-ol^5]-enkephalin, DPDPE = D-Pen2, D-Pen5-enkephalin, ddY-mice = Deutschland, Denken, and Yoken mice, U69593 = (5α,7α,8β)-(+)-N-Methyl-N-[7-(1-pyrrolidinyl)-1-oxaspiro[4.5]dec-8-yl]-benzeneacetamide

Matsumoto et al. (2005b) hypothesised that vas deferens contractility is due to the blockade of neuronal calcium channel which then cause inhibition of norepinephrine and ATP release. Mitragynine inability to inhibit KCl-induced myogenic contraction shows that the inhibitory activity is probably at neuronal levels (Matsumoto et al., 2005b).

(i) Effects on uterus and urinary bladder smooth muscle

Table 2.3i shows a study examined the effect of MS on uterus and urinary bladder contractility. This study used mitragynine to see the changes in contractility activity. There was no significant effect in contractility in both uterus and urinary bladder. In human, there is no report on the effect of MS on the uterus and urinary bladder.

Table 2.3 (i): Pharmacological properties of *Mitragyna speciosa*: Effects on uterus and urinary bladder smooth muscle

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Grewal, 1932a	Guinea-pig (strain not specified)	**Uterus contraction* Mitragynine dilution 1:20000, 1:100,000	Oxytocic drug (not specified) Ringer’s solution	Contractility	Mitragynine has no effect on uterus contractility. However, if induced by oxytocic drug, mitragynine induced immediate relaxation.
	Rabbit (strain not specified)	**Bladder contractility test* Mitragynine dilution 1:20000, 1:100,00	Ringer’s solution	Contractility	Mitragynine caused no effect on bladder contractility.

*test using organ bath

(j) Inflammation

Table 2.3j shows two studies investigated the inflammatory properties of MS. Both studies used MS extract (Raja Aziddin et al., 2005; Mossadeq et al., 2009a). The tests used were carrageenan-induced paw oedema test and cotton-pellet-induced granuloma test.

All studies showed significant effects in analgesia and reduction in inflammation. The mechanism of anti-inflammatory activity of MS could probably be due to combination of inhibition of pro-inflammatory mediator release and vascular permeability (Mossadeq et al., 2009a). The presence of alkaloids, saponins, flavonoids, tannins and sterols in MS may also contribute to the anti-inflammatory effect (Mossadeq et al., 2009a).

Table 2.3 (j): Pharmacological properties of *Mitragyna speciosa*: Inflammation

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Raja Aziddin et al., 2005	Sprague-Dawley rats	*Carrageenan-induced Paw Oedema test* MS extract 50 mg/kg i.p.	Acetylsalicylic acid Vehicle	Changes in paw volume.	MS extract reduced oedema and inflammation.
Mossadeq et al., 2009a	Balb C mice Sprague-Dawley rats	*Paw Oedema and cotton-pellet-induced granuloma test* MS methanol extract 50-200 mg/kg i.p.	Acetylsalicylic acid Morphine Naloxone	Latency for pain. Volume of oedema.	MS methanol extract reduced inflammation on paw oedema and inhibited the growth of granuloma tissue.

i.p.= intraperitoneal, p.o.= oral

(k) Cardiovascular

The Table 2.3 (k) shows two studies examined the cardiovascular effects of mitragynine (Grewal, 1932a; Macko et al., 1972) using cat, dog, frog and rabbit. Macko et al. (1972) used codeine as control.

Grewal (1932a) found that mitragynine reduced blood pressure by dilating the blood vessel. The dilated coronary vessels allow the blood from the aorta to flow to the right auricle that leads to increase pulmonary pressure and respiration. However Macko et al. (1972) found the reduction of blood pressure in cats was minimal except if the dose was increased which can lead to respiratory failure.

In human, there are several anecdotal reports of MS used for hypertension. This could probably be due to the presence of rhynchophylline, a compound that is known to have antihypertensive effects (Shi et al., 2003).

Table 2.3 (k): Pharmacological properties of *Mitragyna speciosa*: Cardiovascular

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Grewal, 1932a	Cat, frog and Rabbit (strain not specified)	*Cardiovascular test.* Mitragynine 2.5-5 mg i.v.	Ringer's solution	Blood pressure Respiration rate	Mitragynine dilated blood vessels in cat, frog and rabbit which then reduced blood pressure and later on increased respiration.
Macko et al., 1972		*Cardiovascular Test.*			
	Dog (strain not specified)	Mitragynine 0.092-9.2 mg/kg i.v.	Codeine	Blood pressure	Minimal alteration to mean arterial pressure (<20 mm/Hg)
	Cat (strain not specified)	Mitragynine 0.46-4.6 mg/kg i.v.	No control	Blood pressure	Minimal hypotensive effect. However, the effect increased with dose. At 4.6 mg/kg cat died due to respiratory failure.

i.v.= intravenous

(l) Antitussive

Macko et al. (1972) studied the effect of mitragynine on dog for its antitussive properties (Table 2.3l). This is the only study on MS to examine antitussive properties. Codeine was used as control. Mitragynine and codeine were reported to be approximately equipotent in suppressing the cough reflex. However mitragynine did not cause emesis like codeine.

Table 2.3 (l): Pharmacological properties of *Mitragyna speciosa*: Antitussive

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Macko et al., 1972	Dog (strain not specified)	*Antitussive Test.* Mitragynine 0.9-9.2 mg/kg i.v.	Codeine	Cough inhibition	Mitragynine and codeine were approximately equipotent in suppressing the cough reflex; however mitragynine did not cause emesis like codeine.

i.v.= intravenous

(m) Hypoglycaemia

Hypoglycaemic effect was briefly examined in Macko et al. (1972) (Table 2.3m). Mitragynine as compared to codeine did not affect blood sugar level in guinea pig.

Purintrapiban et al. (2011) demonstrated the effect of MS in stimulating glucose transport in muscle cells. It was proposed that MS increased glucose transport activity in rat L8 myotubes which subsequently increases the key enzymes activities that are responsible for insulin-stimulated glucose transport. It is worth noting that MS has also been used by the villagers for its antidiabetic properties (Assanangkornchai et al., 2007). However, data are lacking to support routine use of MS as antidiabetic agent.

Table 2.3 (m): Pharmacological properties of *Mitragyna speciosa*: Hypoglycaemia

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Macko et al., 1972	Guinea-pig (strain not specified)	*Hypoglycaemic test* Mitragynine (dose and route of administration not specified)	Codeine	Blood glucose level.	Mitragynine failed to affect blood sugar in contrast with codeine that produced slight hypoglycaemic activity.

i.v.= intravenous

(n) Gastric acid secretion

Tsuchiya et al. (2002) investigated the inhibitory effects of mitragynine on gastric acid secretion (Table 2.3n) in rats with morphine as control. The 2-deoxy-D-glucose was used to induce gastric acid secretion. Acid output was assessed. Mitragynine was shown to inhibit gastric acid secretion via activation of opioid receptors of central nervous system.

It has been thought that the anorexia and weight loss associated with MS might be due to mitragynine inhibiting gastric acid secretion. However, other mechanisms have been proposed: direct inhibition of neurons in the lateral hypothalamus (King et al., 1979; Sikdar and Oomura, 1985); or weight loss associated with an addictive disorder (Mercer and Holder, 1997).

Table 2.3 (n): Pharmacological properties of *Mitragyna speciosa:* Gastric acid secretion

Author(s), year	Animal	*Test* Compound tested, dose and route of administration	Control	Measurement	Main result
Tsuchiya et al., 2002	Wistar rats	*Test on inhibitory effect of mitragynine on gastric acid secretion* Mitragynine 3-30 µg i.c.v.	Morphine Naloxone Vehicle	Acid output.	Mitragynine inhibited gastric acid secretion like morphine via activation of opioid receptors in central nervous system.

i.c.v.=intracerebroventricular

2.3.5 Limitations and Strengths

The methodology for systematic reviews of animal studies is still under development. This study highlights the differences in various aspects of animal studies assessing the pharmacological properties of MS. The studies vary in terms of animal species, MS constituents tested, model used to assess activity, and outcome or measurement used. These variations make it difficult to make a firm conclusion on each of the pharmacological properties of MS.

The strengths of this study was, that this study involved a systematic and comprehensive literature search to identify all possible relevant studies with rigorous, explicit and clearly documented methodology and assess the methodological quality of the included studies. Our search strategies enabled us to locate relevant studies on electronic databases. Meta search engine used such as Google scholar and Scirus has lead us to additional relevant studies that was sometime beyond the standard electronic databases. We also hand searched journals for unpublished data and contacted researchers to identify additional studies.

Identifying the relevant literature was done thoroughly and systematically, however, we could have missed some relevant studies due to unpublished data or studies published in languages other than English. This is the main limitation of a systematic review as a sensitive and advanced searches design might still not be able to trace all relevant studies (Whiting et al., 2008).

2.3.6 Recommendations for Future Research

Given the importance of animal study for providing baseline data on pharmacological properties of any substance, it is important to assess the quality of the study. A standard tool that is able to assess the quality and guide the scientist to conduct proper research to provide valid data is urgently required. Valid data from systematic review allows data on animal studies to be used in further research involving human.

2.4 CONCLUSION

In summary MS has been shown to exert antinociceptive activity in animal models through opioid receptor as well as involving both descending noradrenergic and serotonergic systems by either affecting the biogenic amines levels or modulating the receptors. The postulated mechanisms explain many pharmacological effect associated with MS effects such as antidepressive effects, withdrawal and effect on sleeping. Studies on reduction of smooth much contractility and modulating immune response to reduce inflammatory activity is still under investigation. The pharmacological investigation on MS compounds seems to explain some of the anecdotal effects reported in human.

3. PART 2: *MITRAGYNA SPECIOSA* USE IN THE NORTHERN STATES OF MALAYSIA: A CROSS-SECTIONAL SURVEY

3.1 INTRODUCTION

Compared to Khat, a plant that also has psychoactive effects; little is known about the epidemiology of MS use. Stefan and Mathew (2005) estimated that worldwide about ten million people use Khat on a daily basis while there is few published information on the prevalence of MS use. A cross sectional national survey conducted among youths in Malaysia found that of all drugs abused, 0.5% involved the use of MS and it is mainly abused in the northern states of Peninsula Malaysia (Nazar Mohamed et al., 2008). It appears that MS remains a substance associated with the rural areas.

The present study therefore aimed to describe the use of MS including patterns of use and its effects and to explore its addictive potential among a representative population sample of users in Kedah and Perlis. The need to document the use of MS, its effects and abuse potential is important because over the past decade there has been growing interest in MS as evidenced by the number of advertisements for its sale on the internet (Schmidt et al., 2011). Given its increasing popularity on the internet, it is possible that abuse of MS could spread worldwide.

3.2 MATERIALS AND METHODS

3.2.1 Study design and sampling

The study was conducted between June and September 2010 in the border towns of Kedah and Perlis in the Northern States of Malaysia. Sampling was based on a convenience and snowball sampling method. Adults who had used MS at least once during the past 12 months were recruited. We trained Privileged Access Interviewers (PAIs) to conduct the interviews. The rationale for using a PAI approach is that engaging users from a similar social and cultural background as the target sample increases the recruitment of participants (Elliott et al., 2002). Previous study of the same nature has shown this approach to be effective (Griffiths, 1998). Two trained PAIs conducted face-to-face interviews using a structured questionnaire. The responders were assured of the voluntary nature of the survey and the confidentiality of their responses. All responders provided verbal informed consent.

3.2.2 Questionnaire

Since all the respondents understood the national language of Malaysia (Bahasa Malaysia), the questionnaire, which was written in English **(Appendix F)**, was translated into Bahasa Malaysia **(Appendix G)**. In order to check for equivalence (Herdman et al., 1997) between the two versions, we engaged a translator to translate the translated Bahasa Malaysia questionnaire back to English to confirm that the meaning was similar to the original. The process of translating was repeated until the back translation questionnaire was almost identical to the original.

Demographic data collected included age, occupation, marital status and level of education. Other information collected were information on drug use history, route of MS administration, effects of MS use, and any potential problems experienced associated with MS use. We explored the likelihood that MS could cause dependence by adapting ten questions from Drug Abuse Screening Test-10 (DAST-10) (McCabe et al., 2006). We retained all the wordings of DAST-10, except that we changed the word "drugs" to "Ketum" (the local name for MS) as we were focusing on the use of MS. We excluded DAST-10 scoring for measuring addictive tendencies because it was not our aim to measure the possible abuse of MS; rather we sought to provide preliminary data on the possibility of dependence on MS.

The first draft of the questionnaire was pre-tested on 40 respondents. We used information from the pilot study to modify the questionnaire and the data were not included in the final analysis.

3.2.3 Data analysis

The data was analysed using SPSS version 17. Descriptive statistics such as percentages and means were used to describe the sample on the various variables. Differences between groups were assessed either with an independent t-test or χ^2 test. To measure the internal consistency of DAST-10, Cronbach's α coefficient was calculated. A probability of $p < 0.05$ was considered statistically significant.

3.3 RESULT

3.3.1 Characteristics of the sample

A total of 530 respondents out of 562 subjects approached (91% response rate) agreed to participate in the study. Except for two females, all the respondents were adult males. The demographic characteristics of the samples are as shown in Table 3.1. Almost all the respondents were Malay. About 94% of the respondents used MS on a daily basis. A Chi-squared test for independence was used to determine whether daily use was associated with other demographic variables and showed that except for education level there was no association between daily use with any of the demographic variables examined; marital status, education level, occupation and age groups.

The majority of the respondents (96%) did not use other substances of abuse. However, 22 respondents (4.2%) used MS together with marijuana, amphetamine, heroin or cough syrup.

Table 3.1: Demographic characteristics of the study group

	Daily User		Non-Daily User		Total	
	Number	Percent	Number	Percent	Number	Percent
Gender						
Male	494	93.6	34	6.4	528	99.6
Female	2	100.0	0	0.0	2	0.4
Marital Status						
Married	344	94.0	22	6.0	366	69.1
Single	139	92.1	12	7.9	151	28.5
Divorced	13	100.0	0	0.0	13	2.5
Ethnicity						
Malay	488	93.5	34	6.5	522	98.5
Chinese	5	100.0	0	0.0	5	0.9
Indian	3	100.0	0	0.0	3	0.6
Education level						
Primary and lower secondary	49	89.1	6	10.9	55	10.4
Upper secondary and Tertiary education	447	94.1	28	5.9	475	85.8
Occupation						
Technician and associate professional	15	100.0	0	0.0	15	2.8
Service and sale worker	165	95.9	7	4.1	172	32.5
Skilled agriculture, forestry and fishery workers	168	92.8	13	7.2	181	34.2
Labourers	122	92.4	10	7.6	132	24.9
Unemployed	26	86.7	4	13.3	30	5.7

3.3.2 Usage patterns

The mean duration of MS use was 4.6 (SD = 3.8) years while the mean age of starting MS was 28.3 (SD = 8.1) years. MS was mainly used in a form of a drink (90%), while other methods of consumption including, chewing, smoking and adding the leaves to food constituted the other 10%. The mean effects of MS were reported to last 3.5 (SD = 2.6) hours.

The majority of the respondents obtained the MS from trees grown in the village and a small percentage (13%) bought MS from either coffee shops or restaurants in their village. The frequency of MS use ranged from one to ten times daily. Two thirds of the respondents took MS one to three times daily, with about a quarter taking MS four to six times a day. Very few respondents (2%) took MS more than seven to 10 times a day. Another group of respondents (6%) used MS only when required for minor ailments such as diarrhoea and fever.

3.3.3 Reasons for Use

Many respondents gave more than one reason for using MS. It was mainly used for stamina and endurance, social and recreational use, and improving sexual performance (Fig.3.1). MS was also used to help with sleeping and to reduce withdrawal symptoms and for its euphoric effects. The use for medicinal purposes which included use for alleviating pain, treating diarrhoea, fever and also for diabetes and hypertension constitutes about one third of the total use of MS.

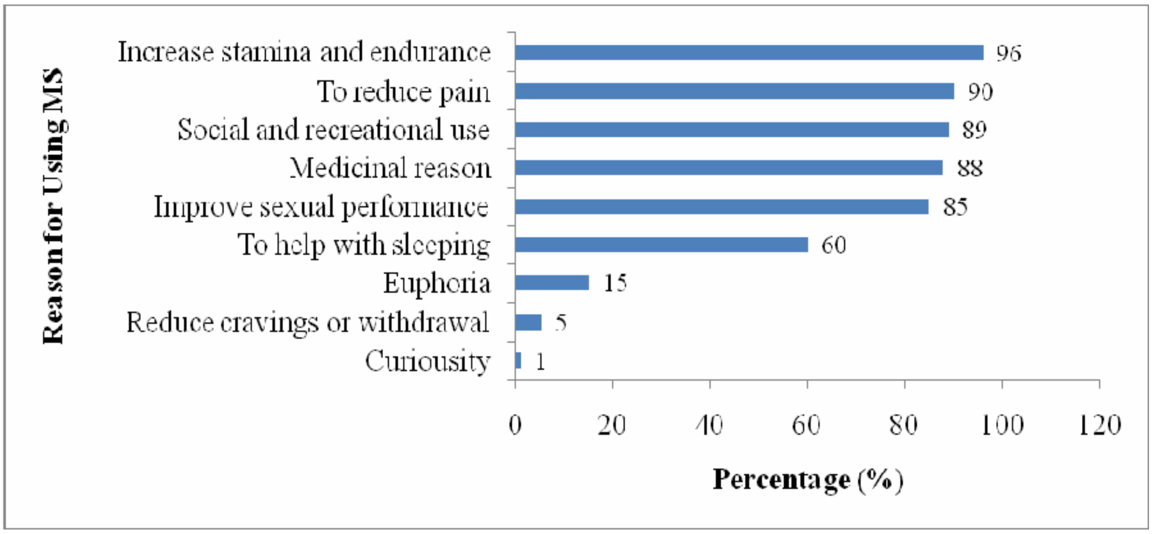

Fig.3.1: Reason of using MS, N=530

3.3.4 Effects of *Mitragyna speciosa* and effects experienced on stopping its use

Table 2 shows a variety of effects were reported with the use of MS. Slightly more than a quarter of the effects reported concerned feeling energised and increased alertness. Another quarter of the effects reported involved euphoric effects such as feelings of relaxation and contentment. There were also reports of hyperpigmentation (10%) particularly on the face. Other effects reported included improved sexual performance, vomiting, feeling sedated and, feeling hot and sweaty. All the effects lasted between an hour and six hours.

The users reported experiencing some unpleasant effects on stopping MS use. The most common effects reported were feeling tired, excessive tearing and jerky movement of limbs. Effects such as hostility and aggression were less commonly reported.

Table 3.2: Effects of MS and effects experienced on stopping MS use

	n	Percentage (%)
Effects reported (total responses=3627)		
Energised and increased alertness	1002	27.6
Euphoria, feeling relaxed and contented	947	26.1
Light headed	102	2.8
Sedated	21	0.6
Hot and sweaty	492	13.6
Improved sexual performance	449	12.4
Hallucination/Induce fantasy	151	4.2
Pigmentation	458	12.6
Others (e.g. vomiting, increased appetite, and bitter taste)	5	0.1
Effects experienced on stopping MS use (total responses=1321)		
Hostility	6	0.5
Aggression	12	0.9
Excessive tearing	396	30.0
Inability to work	51	3.9
Aching of muscles and bones	91	6.9
Jerky movement of limbs	166	12.6
Loss of appetite	17	1.3
Weight loss	17	1.3
Insomnia	59	4.5
Malaise	434	32.9
No effects	64	4.8
Restlessness	8	0.6

N.B: Respondents could give more than one response

3.3.5 Dependence on *Mitragyna speciosa*

Table 3.3 shows that the majority of the respondents (87%) were unable to stop using MS when they wanted to. A very high percentage of users (94%) did not feel guilty about their use of MS. Nevertheless, about 60% of the respondents reported that their family did not agree with their MS use. However, a very high percentage (99%) claimed that they did not neglect their family while using MS.

Table 3.3: Dependence on *Mitragyna speciosa* (with modified DAST-10)

Questions	**N (%)**	
	Yes	**No**
Have you used Ketum other than for medical purposes?	435 (82.1)	95 (17.9)
Do you abuse more than one drug at a time?	24 (4.5)	506 (95.5)
Are you always able to stop using Ketum when you want to?	70 (13.2)	460 (86.8)
Have you had a "blackout" or "flashbacks" as a result of Ketum use?	12 (13.2)	518 (86.8)
Do you ever feel bad or guilty about Ketum use?	31 (5.8)	499 (94.2)
Does your spouse (or parents) ever complain about your involvement with Ketum?	311 (58.7)	219 (41.3)
Have you neglected your family because of your use of Ketum?	7 (1.3)	523 (98.7)
Have you engaged in illegal activities in order to obtain Ketum?	6 (1.1)	524 (98.9)
Have you ever experienced withdrawal symptoms (felt sick) when you stop taking Ketum?	416 (78.5)	114 (21.5)
Have you had a medical problem as a result of your Ketum use (e.g. memory loss, hepatitis, convulsions, bleeding, etc.)?	6 (1.1)	524 (98.9)

3.3.6 The association between socio-demographic variables and the ability to stop MS use

A Pearson Chi-square test conducted to examine whether there was a relationship between the socio-demographic variables (gender, race, marital status and education levels) and the ability to stop using MS revealed that only education level was significantly associated with the ability to stop using MS (χ^2 = 31.0, df=1, p <0.001). A significantly lower proportion of those with upper secondary education and tertiary education (10%) were able to stop using MS compared to those from the primary and lower secondary education (38%).

3.4 DISCUSSION

This study aimed to describe the use of MS among users in the Northern States of Malaysia. The majority of MS users primarily use MS to increase physical endurance for sustained work and most of them were unable to stop using it.

Our sample consisted mainly of male respondents and had only two female respondents. The low number of female respondents was expected as MS is not commonly used among females (Suwanlert, 1975; Tanguay, 2011). The under-representation of females also reflects the general reluctance of women to participate in surveys of this nature (Moore et al., 2010; Vicknasingam et al., 2010). Additionally, MS is mainly used in rural areas in which culturally, society would frown upon its use by females (Suwanlert, 1975).

The majority of the users were using MS on a daily basis. This is not surprising as MS is freely available because of the plentiful supply in villages and surrounding areas. Subjects may also simply buy MS from local coffee shops, since it is being sold openly despite being illegal. The measure chosen by the Government to control MS use by banning mitragynine-containing products can be considered to be ineffective, since cultivation of the trees is still widespread.

It is also important to note the different methods of using the leaves between the Thai and Malaysian users. It appears that in Thailand the leaves are commonly chewed or the leaves are ground before being swallowed with a glass of warm water or other hot beverages like coffee or tea (Assanangkornchai et al., 2007; Suwanlert, 1975) while in our study in Malaysian users, the leaves are mostly boiled and drunk as tea. It would be interesting to find out from clinical pharmacokinetic studies whether the effects of MS are different with the various modes of consumption.

Our findings are in agreement with at least two Thai studies (Suwanlert, 1975) which show that MS is mainly used by labourers who work long hours in the sun. However, a small proportion of respondents use MS to help them with sleeping. It seems that MS has both stimulant and sedative effects. Currently, the stimulating effect of MS has only been reported in humans (Jansen and Prast, 1988b). Apryani et al. (2010) and Reanmongkol et al. (2007) reported that mitragynine has no central stimulant effects in animal models. In a mouse model of depression, MS was shown to have antidepressive effects and no stimulating effects like amphetamine (Farah Idayu et al., 2011). In addition to increasing stamina, MS was also used for reducing pain, which was also reported in two other studies (Assanangkornchai et al., 2007; Vicknasingam et al., 2010). Several studies in animals have shown MS to have antinociceptive effects (Matsumoto et al., 1996a; 1996b; 2006; 2004; Thongpradichote et al., 1998) which involve both the adrenergic and serotonergic systems (Matsumoto et al., 1996b; 1997). These animal data may perhaps provide some evidence that MS could be useful for pain in humans. However, without any proper clinical studies, it is still too early to acknowledge the therapeutic potential of MS for pain relief. Our findings are similar to a Thai Study (Assanangkornchai et al., 2007) in that a small minority of responders reported the use of MS for diabetes and hypertension. Currently, the data from this study and other studies (Assanangkornchai et al., 2007) are insufficient to support the claims for these benefits. In contrast to the findings of Suwanlert (1975) and

Assanangkornchai et al. (2007); our data showed that MS was also used to improve sexual performance by prolonging coitus. It could be possible that MS exerts its effects on the vas deferens as one animal study showed that MS relaxes the smooth muscles of the vas deferens (Horie et al., 2005).

Despite the vast information on the effects of MS, little is known on its side-effects and toxicological properties. Side-effects reported such as light headedness, vomiting and hyperpigmentation are similar to other studies and are reported to be mild (Assanangkornchai et al., 2007; Grewal, 1932; Marcan, 1929; Suwanlert, 1975). Only one study reported serious cases of psychosis associated with the use of MS (Suwanlert, 1975). A high proportion of respondents experienced withdrawal symptoms on stopping MS use. Typical effects experienced were mainly malaise, excessive tearing, and jerky movement of limbs, which are not life threatening and these effects were similar to a Thai study (Assanangkornchai et al., 2007).

Responders with higher education levels were less able to stop using MS compared to those with lower education levels. Our results are inconsistent with the general belief that people with lower education levels might have a tendency to problem-prone behaviour and thus likely to be involved in substances of abuse (Battin-Pearson et al., 2000; Fergusson et al., 2003; Townsend et al., 2006). The probable explanation for this observation is that most of those with lower education levels tend to be unskilled labourers and they use MS mainly to enable them to endure hard physical work, while those with higher education levels use MS for its euphoric effects.

DAST-10 is a brief screening instrument used to assess possible abuse of drugs (McCabe et al., 2006). Since MS has not been established as a drug of abuse, we did not attempt to use DAST-10 to screen for possible abuse to MS. Instead we used DAST-10 for its relevant questions that help to describe qualitatively the social impact of MS on MS users. Despite the reported use of MS to manage withdrawal from other substances of abuse, many respondents reported difficulty in withdrawing from MS, which indicate a possible dependence associated with its use. A further qualitative study may therefore be useful to explore the psychological and social impacts of its use.

We used convenience and snowball sampling methods in recruiting respondents and this may limit the generalisability of our findings to other cultural settings as our respondents were mostly of the Malay race. It is known that the main disadvantage of snowball sampling method is that participants tend to recruit further participants through their own social networks, which might limit the heterogeneity of the sample (Faugier and Sargeant, 1997). Nevertheless, we believe that snowball sampling was the most appropriate method in identifying hidden population of MS users and it was cost effective (Faugier and Sargeant, 1997; Lopes et al., 1996). Additionally, it is also generally known that MS can be considered as a medicinal plant culturally associated more with the Malays (Ong and Nordiana, 1999).

3.5 CONCLUSION

The overall picture was that most users were using MS in a moderate way in terms of frequency for its stimulating effects and drinking the extract was usually a social activity. The majority of users reported one or more desirable effects from MS consumption. There was large number of users who reported their inability to stop using it and closed to 80% of the respondent reported undesirable effects which included excessive tearing, malaise and jerky movements of the limbs on stopping MS use. These users may need some help and support in controlling their MS use. Further research would therefore be useful to explore the psychological and social impacts of the MS use.

4. GENERAL CONCLUSION

The systematic review on the pharmacological properties shows variations in the 44 studies in terms of methodology, the constituents examined and animal strain used. This review highlights the gap in the research on MS particularly in its pharmacology.

Mitragynine is the most abundant alkaloids in MS while 7-hydroxymitragynine is the most potent even though it only presents in a small fraction in MS. Both compounds are postulated to produce the largest pharmacological effects. Multiple pharmacological actions have been observed in animal on the central nervous system, smooth muscles and inflammatory effects. Some of the pharmacological properties showed in animal studies support reports and claims of MS effects in human. MS seems to have considerable analgesic effect and various effects on nervous systems both in animal and human that shows some potential to be used in medical field. However, reports of the effects are sometimes contradictory, and there is little data to make a firm conclusion on its mechanism of action. This requires further research.

The majority of MS users seek its stimulatory effects and its use are reported to be normal during social activity. Despite its wide spread use, there are lack of evidence of safety in human, thus the use of MS as traditional medicine should not be supported until more data are available. Many users admitted to their inability to stop using MS which raise a concern on addiction despite its traditional reported use to wean addicts from opiate addiction. Therefore, addictive tendencies need to be further investigated by using proper tools.

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6. APPENDICES

Appendix A: List of database and sources searched

Source	Beginning year for searches until Feb 2012
Electronic Database	
ABI/INFORM @ProQuest®	1923
Annual Reviews	1930
Academic Source Premier @EBSCOhost	1975
Cochrane @EBSCOhost	1975
CINAHL @EBSCOhost	1975
International Pharmaceutical Abstracts (IPAB) @EBSCOhost	1975
Medical Database @EBSCOhost	1975
PsycARTICLES®@EBSCOHost	1975
Bioline International	1989
BioLit	2008
BioMed Central	1999
BioOne Full-Text	1998
Directory of Open Access Journals (DOAJ)	2003
NextBio	1965
Pubmed	1946
MEDLINE @Ovid	1946
Biological Abstracts® @Ovid	1998
EBM Review	1991
Web of Science	1980
Tripdatabase	1946
Online publishing site search	
Cambridge Journals Online	1700
Library Literature & Information Science Full Text @WilsonWeb	1984
Open-J Gate	2006
Oxford University Press Journals	1849
Sabinet's Full Open Access Journal Collection	2008
Scopus™	1966
SpringerLink	2005
ScienceDirect®	1823

Appendix A, continued

Source	Beginning year for searches until Feb 2012
Nature.com@Nature Publishing Group (NPG)	1869
Specialised electronic journals or websites	
GLOBinMED	2003
MyAIS: Malaysian Abstracting and Indexing System	2007
Networked Digital Library of Theses and Dissertations (NDLTD)	1994
Alternative and Complimentary medicine	1980
Journal of Medicinal Plant Research	2007
Journal of Complimentary and Integrative Medicine	1999
Songklanakarin Journal of Science and Technology	1978
Science Magazine (AAAS)	1997
Hand searches	
Malaysian Journal of Science	2002
Planta Medica	1960-2000
Proceeding of the Alumni Association, Malaya.	1957
Proceedings of 2nd Meeting of the Natural Products Research Group. Chemistry Department Universiti Kebangsaan Malaysia	1985
Proceedings 3rd Medical Colloquium. Universiti Kebangsaan Malaysia	1992

Appendix B: Search Strategies

Database: Ovid

Category	Search Term
#1	Kratom OR ketum OR biak-biak OR Kakuam OR Ithang OR Thom OR *Mitragyna speciosa* OR mitragyna Or mitragynine OR 7-hydroxymitragynine OR mitragynine pseudoindoxyl OR speciogynine OR speciociliatine OR paynantheine OR 7-alpha-hydroxy-7H-mitragynine OR mitragynaline OR pinoresinol OR mitralactonal OR mitrasulgynine OR corynantheidaline OR mitragynalinic acid OR corynantheidalinic acid OR speciofoline OR rhynchophylline OR stipulatine OR ajmalicine OR payantheine OR corynantheidine OR isomitraphylline OR corynoxine A OR mitrafoline OR isomitrafoline OR isospeciofoline OR 3-dehydromitragynine
#2	Animal* OR animal research* OR animal stud* OR animal experiment* OR animal trials OR animal model OR rat OR murine OR mice OR primates OR guinea pig OR hamster OR dog
#3	Animal* OR rat OR murine OR mice OR primates OR guinea pig OR hamster OR dog OR Pharmacology OR antinociceptive OR pain-killer* OR analgesic* OR nerve OR nervous OR neuron* OR opiate* OR receptor OR side-effect* OR skin OR mental disorders OR psychiatry OR psychosis OR strength OR addict* OR sex* OR coitus OR thin OR anorexia OR hepatic face OR dry mouth OR xerostomia OR micturation OR constipation OR diarrhoea OR faeces OR melena OR hostility OR aggressi* OR tears OR flu OR work OR muscle OR myalgia OR bones OR arthralgia OR limb* OR ataxia OR opiate* replacement OR withdrawal OR dependence OR seizure* OR alert* OR fever OR antipyretic OR malaria OR rhino rhea OR insomnia OR concentration OR constricted
#4	#1 AND #2 AND #3

Databased: Science Direct®

Category	**Search Term**
#1	(Kratom OR ketum OR biak-biak OR Kakuam OR Ithang OR Thom OR {*Mitragyna speciosa}* OR mitragyna Or mitragynine OR {7-hydroxymitragynine} OR {mitragynine pseudoindoxyl} OR speciogynine OR speciociliatine OR paynantheine OR {7-alpha-hydroxy-7H-mitragynine} OR mitragynaline OR pinoresinol OR mitralactonal OR mitrasulgynine OR corynantheidaline OR {mitragynalinic acid} OR {corynantheidalinic acid} OR speciofoline OR rhynchophylline OR stipulatine OR ajmalicine OR payantheine OR corynantheidine OR isomitraphylline OR {corynoxine A} OR mitrafoline OR isomitrafoline OR isospeciofoline OR {3-dehydromitragynine})
#2	(Animal? OR {animal research?} OR {animal stud?} OR {animal experiment?} OR {animal trial?}OR {animal model} OR rat OR murine OR mice OR primates OR {guinea pig} OR hamster OR dog)
#3	(Pharmacology OR antinociceptive OR pain-killer? OR analgesic? OR {central nervous system} OR neuron? OR opiate? receptor OR side-effect? OR {dark skin} OR {mental disorders} OR psychiatry OR psychosis OR strength OR addict? OR {sex? Intercourse} OR coitus OR {sex? Performance} OR {sex? Endurance} OR thin OR anorexia OR {hepatic face} OR {dry mouth} OR xerostomia OR {frequent micturation} OR constipation OR diarrhoea OR {small faeces} OR {black faeces} OR melena OR hostility OR aggressi? OR tears OR {wet nose} OR flu OR {inability to work} OR {aching in the muscle} OR myalgia OR {aching in the bones} OR arthralgia OR {jerky movement of limb?} OR ataxia OR {opiate? replacement} OR withdrawal OR dependence OR seizure? OR alert? OR fever OR antipyretic OR malaria OR rhino rhea OR insomnia OR {poor concentration} OR {constricted effect})
#4	#1 AND #2 AND #3

Databased: Web of Science®

Category	**Search Term**
#1	Kratom OR ketum OR biak-biak OR Kakuam OR Ithang OR Thom OR *Mitragyna speciosa* OR mitragyna Or mitragynine OR 7-hydroxymitragynine OR mitragynine pseudoindoxyl OR speciogynine OR speciociliatine OR paynantheine OR 7-alpha-hydroxy-7H-mitragynine OR mitragynaline OR pinoresinol OR mitralactonal OR mitrasulgynine OR corynantheidaline OR mitragynalinic acid OR corynantheidalinic acid OR speciofoline OR rhynchophylline OR stipulatine OR ajmalicine OR payantheine OR corynantheidine OR isomitraphylline OR corynoxine A OR mitrafoline OR isomitrafoline OR isospeciofoline OR 3-dehydromitragynine
#2	Animal* OR animal research* OR animal stud* OR animal experiment* OR animal trials OR animal model OR rat OR murine OR mice OR primates OR guinea pig OR hamster OR dog
#3	Pharmacology OR antinociceptive OR pain-killer* OR analgesic* OR central nervous system OR neuron* OR opiate* receptor OR side-effect* OR dark skin OR mental disorders OR psychiatry OR psychosis OR strength OR addict* OR sex* intercourse OR coitus OR sex* performance OR sex* endurance OR thin OR anorexia OR hepatic face OR dry mouth OR xerostomia OR frequent micturation OR constipation OR diarrhoea OR small faeces OR black faeces OR melena OR hostility OR aggressi* OR tears OR wet nose OR flu OR inability to work OR aching in the muscle OR myalgia OR aching in the bones OR arthralgia OR jerky movement of limb* OR ataxia OR opiate* replacement OR withdrawal OR dependence OR seizure* OR alert* OR fever OR antipyretic OR malaria OR rhino rhea OR insomnia OR poor concentration OR constricted effect
#4	#1 AND #2 AND #3

Appendix C: Data Extraction Form

Data Extraction Form	
Author, Year	
Animal	
Intervention and dosage	
Type of test	
Main result	
Quality of study (CAMARADES)	The answer can be: Yes/No/Unclear
Peer reviewed publication	
Control of temperature	
Random allocation to treatment or control	
Blinded to treatment	
Blinded assessment of outcome	
Appropriate animal model (aged, diabetic, or hypertensive)	
Sample size calculation	
Compliance with animal welfare regulations	
Statement of potential conflict of interests	

Appendix D: List of Criteria in CAMARADES

Items	**Meaning**
Peer-reviewed publication	Work, research, or ideas to the critically appraised by experts in the same field, before a paper describing this work is published in a journal.
Statement of temperature control	Temperature ± range (regulated or not)
Random allocation to treatment or control	1. Give full details of how animals were allocated to experimental groups, including randomisation or matching if done. 2. A describe order in which the animals in the different experimental groups were treated and assessed.
Blinded to treatment	Keeping the persons who perform the experiment and collect data unaware of the treatment allocation.
Blinded to assessment of outcome	Keeping the persons who assess outcome unaware of the treatment allocation.
Use of anaesthetic without significant intrinsic neuroprotective activity	Declaration that the anaesthetic used is not affecting the experiments (applied only to studies that involved central nervous system)
Appropriate animal model (example: aged, diabetic, hypertensive or healthy)	Using an appropriate animal model in experiments that require specific type of condition (hypertension and diabetes)
Sample size calculation	1. Specify the total number of animals used in each experiment and the number of animals in each experimental group. 2. Explain how the number of animals was decided. Provide details of any sample size calculation used.

Appendix D, continued

Items	**Meaning**
	3. Indicate the number of independent replications of each experiment, if relevant.
Compliance with animal welfare regulations	Description of the ethical and qualitative assessment by an independent organisation within the institute (e.g. Institutional Ethics Committee)es for the care and use of animals.
Statement of potential conflict of interests	List all funding sources (including grant number) and the role of the funder(s) in the study.

Appendix E

Summary of excluded study and reasons for exclusion

Excluded studies

Study	Reason for exclusion
Horie et al., 1995	Cells study.
Watanabe et al., 1999	Chapter from a book. The data was already published elsewhere as papers.
Yamamoto et al., 1999	Synthesised compound of MS.
Matsumoto, 2006	A thesis. The author already published the data elsewhere as papers.
Matsumoto et al.., 2006b	Synthesised compound of MS.
Matsumoto et al.., 2008	Synthesised compound of MS.
Abdul Mukmin, 2011	Unpublished study. Thesis is still not released by the university.

Appendix F

QUESTIONNAIRE ON USE OF KETUM (*MITRAGYNA SPECIOSA*)

Introduction (please read to the patient)
Thank you for agreeing to take part in this brief interview about Ketum. I am going to ask you some questions mainly about your experience of using Ketum across your life time and in the past twelve months. The aim of this study is to explore the use of Ketum and its effects. Please be assured that information on such use will be treated strictly confidential.

Section A: Demographic data

Gender	☐ Male[1]	☐ Female[2]
Age	________________________	
Marital status	☐ Single[1]	☐ Married[2]
	☐ Divorced[3]	☐ Other[4] ________________
Ethnicity	☐ Malay[1]	☐ Chinese[2]
	☐ Indian[3]	☐ Others[4] ________________
Education level	☐ Primary School[1]	☐ Lower Secondary (Form 1-3)[2]
	☐ SPM[3]	☐ STPM[4]
	☐ Degree[5]	☐ Postgraduate[6] ____________
	☐ Other[7] ______________________________________	
Home state	____ (e.g. Kedah, Penang, WP, Sabah, etc.)	
Occupation	____	

Section B: History of Ketum Use

Q8. How old were you when you first started using Ketum? ____________

Q9. Who influenced you to use Ketum?

☐ Parents^{1} ☐ Siblings^{2}

☐ Elders^{3} ☐ Friends^{4}

☐ Dealer^{5} ☐ Others^{6}(*specify*)_______________

Q10. Is Ketum commonly used in your community? ☐ Yes ☐ No

Q11. Who else in your family use Ketum?___________________________________

Q12. With whom do you use Ketum?

☐ Parents^{1} ☐ Siblings^{2}

☐ Elders^{3} ☐ Friends^{4}

☐ Dealer^{5} ☐ Others^{6}(*specify*)______________

Q13. How long did you use Ketum? ______________________________________

Q14. Where did you get your supply of Ketum?______________________________

Q15. How did you normally use Ketum?

☐ As a drink (boiled leaves) ☐ Chewed (leaves)

☐ Smoked (dry leaves) ☐ Swallowed (paste)

☐ Added to food or drink (powdered extract) ☐ Others (*specify*)______

Q16. How often did you use Ketum? *(specify)*________________________

Q17. What are the reasons you used Ketum? *Please tick the boxes.*

a.	To get high	☐
b.	To have more stamina and endurance	☐
c.	To reduce pain	☐
d.	To help with sleeping	☐
e.	Recreational	☐
f.	Social use	☐
g.	To reduce drug withdrawal symptoms	☐
h.	Others (*please specify)*________________	☐

Q18. Do you use other drug (non-medicinal use) other than Ketum? ☐ No ☐ Yes (*specify)*__________

Q19. Do you use Ketum and other drug (non-medicinal use) at the same time? ☐ No ☐ Yes (*specify)*__________

Section C: Effects of Ketum

Q20. Thinking about your usage of Ketum, what effects did you experience?

	Effects	Yes	No
a.	Energized	☐	☐
b.	Euphoria	☐	☐
c.	Stimulated	☐	☐
d.	Sedated	☐	☐
e.	More alert	☐	☐
f.	Comfortable pleasure	☐	☐
g.	Relaxed	☐	☐
h.	Contented	☐	☐

i.	Motivated	☐	☐
j.	Others *(please specify)* ____________________	☐	☐

Q21. How long did the Ketum effects last? ________________________

Q22. Thinking about your usage of Ketum, what effects did you experience when Ketum was not available?

Effects		Yes	No
a.	Hostility	☐	☐
b.	Aggression	☐	☐
c.	Excessive tearing	☐	☐
d.	Inability to work	☐	☐
e.	Aching of muscles and bones	☐	☐
f.	Jerky movement of limbs	☐	☐
g.	Anorexia	☐	☐
h.	Weight loss	☐	☐
i.	Insomnia	☐	☐
j.	Malaise	☐	☐
k.	No effects	☐	☐
l.	Others *(please specify)*____________________	☐	☐

Section D: Drug Use Questionnaire (DAST-10)

		Yes	No
Q23.	Have you used Ketum other than those required for medical reasons?	☐	☐
Q24.	Do you abuse more than one drug at a time?	☐	☐
Q25.	Are you always able to stop using Ketum when you want to?	☐	☐
Q26.	Have you had “blackout” or “flashbacks” as a result of Ketum use?	☐	☐
Q27.	Do you ever feel bad or guilty about Ketum use?	☐	☐
Q28.	Does your spouse (or parents) ever complain about your involvement with Ketum?	☐	☐
Q29.	Have you neglected your family because of your use of Ketum?	☐	☐
Q30.	Have you engaged in illegal activities in order to obtain Ketum?	☐	☐
Q31.	Have you ever experienced withdrawal symptoms (felt sick) when you stop taking Ketum?	☐	☐
Q32.	Have you had medical problem as a result of your Ketum use (e.g. memory loss, hepatitis, convulsions, bleeding, etc.)?	☐	☐

Q33. Comments (about the study and Ketum in general)

____

____

____

Thank you for your cooperation.

Appendix G

SOAL SELIDIK PENGGUNAAN KETUM (*MITRAGYNA SPECIOSA*)

Pengenalan (sila bacakan kepada responden)
Terima kasih kerana bersetuju mengambil bahagian dalam soal selidik kami tentang Ketum. Saya akan menanyakan soalan tentang pengalaman anda menggunakan ketum sepanjang hidup anda dan 12 bulan yang lalu. Tujuan soal selidik ini adalah untuk meninjau penggunaan Ketum dan kesannya. Maklumat yang anda beri akan dirahsiakan.

Bahagian A: Demografi

Jantina ☐ Lelaki^{1} ☐ Perempuan^{2}

Umur ____________________________

Status perkahwinan ☐ Bujang^{1} ☐ Sudah berkahwin^{2}
☐ Bercerai^{3} ☐ Lain-lain^{4} ________________

Bangsa ☐ Melayu^{1} ☐ Cina^{2}
☐ India^{3} ☐ Lain-lain^{4} ________________

Taraf pendidikan ☐ Sekolah rendah^{1} ☐ Menengah Rendah (Tingkatan 1-3)^{2}
☐ SPM^{3} ☐ STPM^{4}
☐ Ijazah^{5} ☐ Pascaijazah^{6} __________________
☐ Lain-lain^{7} ____

Negeri asal ____
(e.g. Kedah, Penang, WP, Sabah, etc.)

Pekerjaan ____

Bahagian B: Sejarah penggunaan ketum

S8. Berapakah umur anda ketika kali pertama menggunakan Ketum? ____________

S9. Siapakah yang mempengaruhi anda menggunakan Ketum?

☐ Ibu bapa[1]
☐ Orang tua/sesepuh[3]
☐ Pengedar[5]

☐ Adik beradik[2]
☐ Kawan[4]
☐ Lain-lain[6](*nyatakan*)________

S10. Adakah Ketum selalu digunakan oleh penduduk sekitar anda? ☐ Ya ☐ Tidak

S11. Siapakah diantara keluarga anda menggunakan Ketum?__________________

S12. Anda menggunakan Ketum dengan siapa?

☐ Ibu bapa[1]
☐ Orang tua/sesepuh[3]
☐ Penjual [5]

☐ Adik beradik[2]
☐ Kawan[4]
☐ Lain-lain[6](*nyatakan*)___________

S13. Berapa lamakah anda mengguna Ketum? _______________________

S14. Di manakah anda mendapat bekalan Ketum anda?________________________

S15. Bagaimanakah anda biasanya menggunakan ketum?

☐ Sebagai minuman (air rebusan daun)
☐ Dihisap seperti rokok (daun kering)
☐ Dicampurkan di dalam makanan dan minuman (pati serbuk)
☐ Dikunyah (daun segar)
☐ Ditelan (giling)
☐ Lain-lain (*nyatakan*)________________________

S16. Berapa kerap anda menggunakan ketum? *(nyatakan)*________________________

S17. Apakah penyebab anda menggunakan Ketum? *Sila tandakan dalam kotak di bawah.*

a.	Mengkhayalkan	☐
b.	Memberi stamina dan ketahanan badan	☐
c.	Mengurangkan sakit pada badan	☐
d.	Memudahkan tidur	☐
e.	Bersantai	☐
f.	Bersosial	☐
g.	Mengurangkan kesan penghentian drug *(withdrawal)*	☐
h.	Meningkatkan ketahanan seks	☐
i.	Kegunaan perubatan (seperti kencing manis, kecacingan, cirit-birit dan sebagainya)	☐
j.	Lain-lain (nyatakan)	☐

S18. Adakah anda pernah mengambil dadah? (seperti heroin, ganja, syabu dan sebagainya) ☐ Tidak ☐ Ya(*nyatakan)*____________

S19. Adakah anda menggunakan dadah tersebut bersama Ketum? ☐ Tidak ☐ Ya(*nyatakan)*___________

Bahagian C: Kesan Ketum

S20. Bila difikirkan tentang pengguna Ketum yang pernah anda gunakan, apakah pengalaman yang anda lalui dari kesan menggunakannya?

	Kesan	Ya	Tidak
a.	Bertenaga	☐	☐
b.	Amat seronok	☐	☐
c.	Pening/berpusing	☐	☐
d.	Rasa mengantuk dan mahu tidur	☐	☐
e.	Lebih segar	☐	☐

f.	Perasaan nikmat	☐	☐
g.	Relaks/Santai	☐	☐
h.	Berasa puas hati/Bahagia	☐	☐
i.	Panas dan berpeluh	☐	☐
j.	Meningkatkan ketahanan seks	☐	☐
k.	Khayal	☐	☐
l.	Lain-lain (nyatakan)_________________________	☐	☐

S21. Berapa lamakah kesan Ketum tersebut bertahan? ____________________________

S22. Memikirkan balik penggunaan Ketum, apakah kesan yang anda alami jika Ketum tidak didapati?

Kesan		Ya	Tidak
a.	Perasaan permusuhan	☐	☐
b.	Agresif	☐	☐
c.	Menguap dan berhingus	☐	☐
d.	Tidak dapat bekerja	☐	☐
e.	Sakit otot dan tulang	☐	☐
f.	Sentapan gerakan anggota badan	☐	☐
g.	Tiada selera makan	☐	☐
h.	Hilang berat badan	☐	☐
i.	Tidak dapat tidur	☐	☐
j.	Rasa tidak sedap badan/dedar	☐	☐
k.	Tiada kesan apa-apa	☐	☐
l.	Lain-lain (nyatakan)__________________________	☐	☐

Bahagian D: ***Drug Use Questionnaire (DAST-10)***

		Ya	Tidak
S23.	Pernahkah anda menggunakan Ketum untuk tujuan selain perubatan?	☐	☐
S24.	Adakah anda menyalahgunakan dadah lebih daripada satu jenis dalam satu masa?	☐	☐
S25.	Adakah anda sentiasa boleh berhenti menggunakan Ketum bila anda menghendakinya?	☐	☐
S26.	Pernahkah anda “pitam/pengsan/tidak sedarkan diri” atau “ teringat perkara yang lepas/*flashback”* akibat penggunaan Ketum?	☐	☐
S27.	Adakah anda merasa teruk atau bersalah menggunakan Ketum?	☐	☐
S28.	Adakah pasangan anda (atau ibu bapa) pernah bersungut /berleter tentang penglibatan anda dengan Ketum?	☐	☐
S29.	Pernahkah anda tidak menghiraukan keluarga disebabkan penglibatan anda dengan Ketum?	☐	☐
S30.	Pernahkah anda melibatkan diri dengan perkara yang melanggar undang-undang untuk mendapatkan bekalan Ketum?	☐	☐
S31.	Pernahkah anda mengalami kesan sampingan (merasa sakit) selepas berhenti menggunakan Ketum?	☐	☐
S32.	Pernahkah anda mengalami masalah perubatan akibat menggunakan Ketum? (contoh: hilang ingatan, hepatitis, sawan, pendarahan dan lain-lain)	☐	☐

S33. Komen (secara am tentang kajian ini dan Ketum)

____

____

____

Terima kasih atas kerjasama anda.

All in-text references underlined in blue are linked to publications on ResearchGate, letting you access and read them immediately.